Chylomicron metabolism in rats: kinetic modeling indicates that the particles remain at endothelial sites for minutes

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Abstract

Chylomicrons labeled in vivo with 14C-oleic acid (primarily in triglycerides, providing a tracer for lipolysis) and 3H-retinol (primarily in ester form, providing a tracer for the core lipids) were injected into rats. Radioactivity in tissues was followed at a series of times up to 40 min and the data were analyzed by compartmental modeling. For heart-like tissues it was necessary to allow the chylomicrons to enter into a compartment where lipolysis is rapid and then transfer to a second compartment where lipolysis is slower. The particles remained in these compartments for minutes and when they returned to blood they had reduced affinity for binding in the tissue. In contrast, the data for liver could readily be fitted with a single compartment for native and lipolyzed chylomicrons in blood, and there was no need for a pathway back to blood. A composite model was built from the individual tissue models. This whole-body model could simultaneously fit all data for both fed and fasted rats and allowed estimation of fluxes and residence times in the four compartments; native and lipolyzed chylomicrons ("remnants") in blood, and particles in the tissue compartments where lipolysis is rapid and slow, respectively.—Hultin, M., R. Savonen, O. Chevreuil, and T. Olivecrona. Chylomicron metabolism in rats: kinetic modeling indicates that the particles remain at endothelial sites for minutes. J. Lipid Res. 2013. 54: 2595–2605.

Supplementary key words adipose tissue • compartmental modeling • chylomicrons • chylomicron remnants • endothelium • heart • lipoprotein kinetics • lipolysis and fatty acid metabolism • lipoprotein lipase • margination

Dietary fat is transported from the intestine in chylomicrons (1, 2). The metabolism of these is considered to be a two-step process (3–5). In the first step triglycerides (TGs) in the chylomicron particles are hydrolyzed by lipoprotein lipase (LPL) (2, 6). This generates TG-depleted particles, chylomicron remnants, which are taken up and degraded in the liver by receptor-mediated endocytosis (7). One approach to study these processes is compartmental modeling, which allows for testing of hypotheses regarding the structure of the system and for calculations of residence times and fluxes (8). In a previous study we used compartmental modeling to study the disappearance of chylomicron lipids from the circulating blood (6). The results indicated that a large fraction of chylomicron TGs leaves plasma with the particles, and that most of the fatty acids from chylomicron TGs mix into the same metabolic compartment as do plasma FFAs. That study was based on data for radioactivity in blood lipids. In the present study we focus on the binding/sequestration of chylomicrons in tissues, and the subsequent fate of the particles and their lipids. This is based on data for radioactivity in blood lipids. In the present study we focus on the binding/sequestration of chylomicrons in tissues, and the subsequent fate of the particles and their lipids. This is based on data for radioactivity in tissues at a series of short times after injection of doubly labeled chylomicrons. The major questions asked were: What is the nature of the interaction of chylomicron particles with the vascular endothelium? Are the interactions short-lived so that there is in effect near-equilibrium between particles in blood and at the endothelium or is the interaction of a chylomicron particle with an endothelial binding-lipolysis site more long-lived? If so, can we estimate the residence time?

EXPERIMENTAL PROCEDURES

Animal procedures

Male Sprague-Dawley rats (150 g), from Moellegaard Breeding Center, Skensved, Denmark, were allowed to acclimatize for at least 1 week after transport before being included in the study. They were allowed free access to water and standard chow and were kept on a 12 h/12 h light/dark cycle with light start at 6:00 AM. At the time of the experiment they weighed 217 ± 13 g (fed)

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and 192 ± 12 g (fasted). The experiments were done between 9:00 AM and noon on two consecutive days. The experiments involved two (fed and overnight fasted) times six groups of five animals for a total of 60 rats.

Animal protection laws required anesthesia and analgesia for the animals. The rats were anesthetized by intramuscular injections of 0.5 ml/kg body weight Hypnorm Vet® (Janssen Animal Health, Stockholm, Sweden; fentanyl citrate, 0.315 mg/ml and fluanisone, 10 mg/ml) and 0.5 ml/kg body weight Dormicum® (Roche, Stockholm, Sweden; midazolam, 5 mg/ml). All animal procedures were approved by the animal ethics committee of northern Sweden.

Preparation of lymph chylomicrons

Labeled lymph chylomicrons were obtained as described by Hultin, Savonen, and Olivecrona (6). Briefly, rats were anesthetized and plastic tubes were inserted into the thoracic duct and into the stomach. The gastric tubing was kept patent by infusion of 2.5 ml/h 5% glucose, 0.85% NaCl, and 0.05% KCl. The rats were allowed to recover overnight. On the following day 150 μCi [11,12-3H(N)]retinol (Amersham, UK) and 150 μCi [1-14C]oleic acid (Amersham) was incorporated into 2 ml 10% Intralipid® (Pharmacia-Upjohn Hospital Care, Uppsala, Sweden) by sonication and then given in the gastric tubing. Labeled lymph was collected from the thoracic duct cannula into tubes containing 0.05% EDTA and 0.01% gentamicin (Garamycin®, Schering Corporation, Kenilworth, NJ) for 5 h. The lymph was cleared from white blood cells by a 10 min low speed centrifugation and then layered under 0.154 M NaCl, 0.05% EDTA, and 0.01% Garamycin and spun for 30 min at 35,000 rpm in a Beckman SW 50 rotor. The isolated chylomicrons were resuspended in the same buffer to a final concentration of 20 mg TGs/ml.

Experimental protocol

At noon the day before the experiment, rats were divided into groups that were either allowed free access to standard chow and tap water (fed) or only tap water (fasted). The next day rats were anesthetized as described above and doubly labeled chylomicrons were injected (0.200 ml, 4 mg TGs) into the exposed right jugular vein. Blood samples (0.2 ml) were taken from the left jugular vein and immediately put into preweighed tubes containing 0.05% EDTA and 0.01% gentamicin (Garamycin®, Schering Corporation, Kenilworth, NJ) for 5 h. The lymph was cleared from white blood cells by a 10 min low speed centrifugation and then layered under 0.154 M NaCl, 0.05% EDTA, and 0.01% Garamycin and spun for 30 min at 35,000 rpm in a Beckman SW 50 rotor. The isolated chylomicrons were resuspended in the same buffer to a final concentration of 20 mg TGs/ml.

RESULTS

Chylomicron clearance and tissue binding/uptake

Twenty minutes after injection of chylomicrons to fed rats, 38% of the core label and 15% of the TG label remained in blood (Table 1). About 42% of the core label but only 19% of the TG label were in the liver. These figures are similar to those in our previous study (6) and agree with the concept that chylomicrons progressively lose TG label as they circulate in blood (6, 10–12), and that the liver takes up a large fraction of the lipolyzed particles (3, 4, 6). The new aspect here is the data at shorter times. All tissues studied showed significant labeling as early as 1 min after injection (Table 1). The contribution of label from chylomicrons in blood remaining in the tissues has been subtracted out.

In the liver there was already 5.9% of the injected core label and 4.6% of the TG label at 1 min, the first time studied. The amount of core label in liver then increased throughout the time studied. Comparing fed and fasted rats, the values for radioactivity in liver were similar up to and including the 13 min time point, but at 20 and 40 min there was less TG label in livers of the fasted rats.
TABLE 1. Tissue distribution of label at a series of times after injection of doubly labeled chylomicrons to fed or fasted rats

| Time  | Fed             | Fasted          |
|-------|-----------------|-----------------|
|       | $^3$H-retinol ester (core label) | $^{14}$C-TG | $^3$H-retinol ester (core label) | $^{14}$C-TG |
|       |                  |                 |                               |              |
|       |                  |                 |                               |              |
|       |                  |                 |                               |              |
|       |                  |                 |                               |              |
|       |                  |                 |                               |              |

Fed rats were allowed free access to standard chow and water while fasted rats were deprived of food for 20 h before sacrifice. The contribution of label from chylomicrons in blood remaining in the tissues has been subtracted out. This was done on the basis of blood volumes in the tissues as measured by $^{51}$Cr-labeled red blood cells in separate rats handled identically (6). The data are expressed as percent of injected radioactivity. Values are given as mean ± SEM. *$P < 0.05$ as compared with the corresponding value between fed and fasted rats (ANOVA).

1. The blood volume was calculated as 5.5% of body weight.

2. Per whole organ. The weights of the tissues were: liver 8.5 ± 1.8 g, heart 0.83 ± 0.06 g, lungs 1.11 ± 0.11 g, spleen 0.66 ± 0.10 g, and kidneys 0.88 ± 0.09 g.

3. Per gram tissue.
In the epididymal fat pad of fed rats, chylomicron core label rose to about 0.8% of the injected dose per gram tissue at 13 min and then decreased slowly. At 1 min, TG label was similar to core label. Then TG label rose above core label and reached about 1.5% per gram tissue at 13 min and remained essentially unchanged to the last time point, 40 min. Both core and TG labels were lower in the fasted than in the fed rats. For instance, at 20 min there was 1.45% of the TG label per gram tissue in the fed rats but only 0.34% in the fasted rats. As a 200 g rat contains 10–15 g adipose tissue, this indicates that at least 15% of the chylomicron fatty acids were deposited in the adipose tissue of fed rats, but less than 5% in fasted rats.

In hearts, the early peak of core radioactivity was more pronounced than in the adipose tissue, and the values were higher in fasted than in fed rats. In the fasted rats, 2.7% of the injected core radioactivity was already in the heart at 1 min. This rose to 4.2 and 4.7% at 3 and 7 min and then decreased again to 1.1% at 40 min. The ratio of TG to core label was already well below that in blood at 1 min, 0.70 compared with 0.86, indicating a rapid hydrolysis of chylomicron TGs in the heart.

In the other tissues studied, the patterns of radioactivity were less distinctive than in liver, adipose tissue, or heart, and there were no pronounced differences with nutritional state. In spleen the pattern was reminiscent of that in liver with a continuous increase of core label to 20 min and a steady level or slow decline thereafter. In the lungs there was an early rise of the core label followed by a slow decrease and TG label fell below core label. This pattern was reminiscent of that in hearts of fed rats, but not as pronounced as that in hearts of fasted rats. The kidneys showed a similar pattern for core label as the lungs, but TG label tended to stay close to the core label.

Compartmental modeling

To further analyze the data, we turned to compartmental modeling. In a previous study we focused on the clearance of chylomicrons from blood and recirculation of chylomicron-derived fatty acids in the plasma FFAs (6). The focus of the present study was to analyze binding of chylomicrons in tissues, hydrolysis of TGs, and return of the particles to blood. Our strategy was to first model each tissue separately and then attempt to fit whole-body chylomicron metabolism as a composite of the tissue models.

In our previous model lipolysis took place in two chylomicron compartments, both of which were assumed to be partly in blood and partly marginated at endothelial binding-lipolysis sites spread over all tissues (6). Our present data for radioactivity in TGs and FFAs in blood can be fitted to this model with adjustment of some rate constants (supplementary Fig. I).

In the previous study, labeled red blood cells were injected with the labeled chylomicrons (6) to estimate blood volume. The distribution volume for injected chylomicrons in the blood compartment was calculated and designated as the fraction in blood, or otherwise expressed, the fraction that had not marginated. The fraction in blood in the previous study was 0.71 ± 0.07 and 0.85 ± 0.04 (mean ± SEM) in fed and fasted rats, respectively (6). In the present study, the corresponding values (supplementary Fig. 1) were in the same range, 0.64 ± 0.09 and 0.72 ± 0.09 (parameter estimate ± SD). In the whole-body model derived later in the article (Figs. 4, 5), the blood compartment distribution volumes are fixed to the blood volume. To account for binding before uniform mixing, e.g., first-pass effects, we have allowed for an “initial condition” of instantaneous uptake of radioactivity into the tissue compartments.

Our models do not account for uptake of label from plasma FFAs. This must be substantial in view of the rather large recirculation of TG label in this form (6, 11, 13). We therefore explored the impact of adding an FFA compartment to our models. For this we used the FFA model from earlier studies (6, 14) and let the rate constants adjust using the present data for label in plasma FFAs. This fit was then used as a forcing function and we explored a range of plausible fractions of the total FFA turnover that entered the tissues we modeled (e.g., one-third in the liver). The modeling process could readily adjust to this by increasing the rate constant for loss of fatty acids through oxidation or transfer back into blood. In conclusion, including FFA recycling did not improve the fits of core and TG labels and the rate constants could not be estimated with any precision.

Derivation of a model for heart

The most distinctive time curves for core and TG labels were in hearts of fasted rats. Therefore, these data were used for building a basal model. We first tried to model the data for core label, i.e., the lipoprotein particles. In the simplest model (supplementary Fig. II), the particles can bind in the heart and return to blood. It was not possible fit the data to this two-compartment model. It was necessary to introduce a change in binding behavior for core label in blood such that the chylomicron particles initially bound avidly in heart but were converted to particles (remnants) that bound much less avidly. With this introduction of a second compartment in blood it was possible to fit core label quite well (supplementary Fig. III).

The next step was to try to simultaneously fit the data for core and TG labels in heart (Fig. 1). This was not possible using the three-compartmental model. TG label already fell much below core label at the first data point, 1 min. This indicates that the incoming chylomicrons are rapidly lipolyzed in compartment qH3 and the fatty acids are lost by oxidation or release back into blood as FFAs. If the chylomicrons/remnants stay in this compartment (qH3), the calculated TG label falls below the experimental data at later times. We have therefore added a compartment (qH4) where lipolysis (and therefore loss of TG label by oxidation or release of fatty acids to blood as FFAs) is much slower (k0,H4 << k0,H3) than in the first heart compartment (qH3). To adequately fit the later time points, a storage compartment for TG label (qH5) was needed. This five-compartmental model generated good fits for core and TG labels for the first 13 min.

Adipose tissue

The data for epididymal adipose tissue core label in fed rats could be fitted to a simplified compartmental model.
derived from the heart model (Fig. 2). The present data could not resolve a need for two separate compartments for chylomicrons in blood for binding to adipose tissue. Also, there was no need for storage of core label in qF2, i.e., all core label could be assumed to return to blood with the lipolyzed chylomicron particles (k_{0,F1}).

The pattern for TG label was quite different in adipose tissue compared with heart. TG label was higher than core label from 2 min, indicating that most of the fatty acids from lipolysis were retained in the tissue by reesterification. Therefore, two compartments for chylomicrons/remnants in adipose tissue could not be resolved, while a compartment for storage of TG label (qF5) was needed. If the rate constants for loss of TG label (k_{0,F3}, k_{0,F5}) were set to zero, as if all fatty acids were retained in the tissue, the model returned good fits up to 13 min. From that time the experimental data for TG label remained at a plateau or even decreased a little. This was best fitted by adding an out pathway from the storage pool (k_{0,F5}) that represents hydrolysis of newly synthesized TGs and release of the fatty acids into plasma, but this rate constant was very low.

Core and TG labels were lower in fasted than in fed rats at all time points, and the time curves were less informative. The data could be fitted to the same models as for fed rats (not shown, but see Fig. 5). The experimental data for perirenal adipose tissue could be fitted to the same model as for epididymal adipose tissue, with some adjustment of the rate constants (not shown).

Liver

The data for core label in liver showed a monotonic increase. One compartment in blood (qB1) and one in the liver (qL1) was enough (Fig. 3). There was no need to separate chylomicrons and remnants and there was no need to have separate compartments for initial binding (qL1) and for storage. This indicates that the liver took up chylomicrons and remnants with similar rate constants and stored almost all of the core label. A pathway out from compartment qL1 was needed (k_{0,L1}) representing loss of retinol from the liver, either by degradation or by transfer back into blood. This rate constant was relatively low, and accounted for loss from the liver of only 5% and 14% of the injected retinol compared with 59% and 47% retained in the liver in fed and fasted rats, respectively.

The rate constant for loss of fatty acid radioactivity by oxidation or by release into blood (k_{0,L3}) was much higher than the analogous rate constant for loss of retinol radioactivity (k_{0,L1}). A storage compartment (qL5) was needed to fit the TG label. This compartment represents fatty acids reesterified into TGs and other lipids.

It has been suggested that chylomicrons must be lipolyzed before they can enter the liver (6, 10, 12, 15, 16). We therefore tried setting uptake of chylomicrons (k_{L1,B1}) to zero such that the liver only took up remnants (k_{L1,B2}, suplementary Fig. IV). This did not fit the data; there was a distinctive lack of both core and TG labels in the liver at the early time points. The low TG label could not be compensated for by including uptake of FFAs into the model (not shown). Hence, the data indicate that chylomicrons

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**Fig. 1.** Binding/uptake in heart in fasted rats. The symbols show the observed data for groups of five rats with their SEM expressed as percent of injected radioactivity per heart. The data were fitted to the model shown in the lower panel. Forcing functions were used to represent the sum of compartments qB1+qB2 and qB3+qB4, which are core and TG labels, respectively, in native and lipolyzed chylomicrons in blood. Compartments qH1 and qH2 are core label in heart and qH3, qH4, and qH5 are TG label in heart. To satisfy the data for TG label it was necessary to introduce a second compartment, qH2/qH4 for chylomicrons/remnants in the tissue. The rate constants for transfer from qB1/qB3 to qB2/qB4 in blood were allowed to adjust in the fitting process while the total content in the two compartments was set by the forcing functions. The transfer to qH2/qH4 from qH1/qH3 was rapid and could not be resolved (but see Fig. 4). It was set to 10 min⁻¹. Circles, core label (³H-retinol); squares, TG label (¹⁴C-oleic acid). The syringe shows where the tracer was introduced or the model fit requested an initial condition. Dashed lines connected to a circle indicate compartments for which experimental data is available. Constraints: k_{H2,B2} = k_{H1,B1}, k_{H2,B4} = k_{H1,B3}; k_{H4,H3} = k_{H2,H1}, k_{H4,B3} = k_{H2,B1}. Derived parameter estimates ± SD for fasted rats: k_{B2,B1} = 0.33 ± 0.33 min⁻¹, k_{B1,B2} = 0.036 ± 0.021 min⁻¹, k_{H1,B1} = 0.0044 ± 0.0040 min⁻¹, k_{0,H2} = 0.14 ± 0.10 min⁻¹, k_{0,H3} = 4.0 ± 2.6 min⁻¹, k_{0,H4} = 0.14 ± 0.07 min⁻¹, k_{0,H5} = 0.011 ± 0.004 min⁻¹.
and remnants enter the liver with similar rate constants, in contrast to the marked difference noted for heart and adipose tissue where remnants showed much reduced binding affinity. Hydrolysis of chylomicron TGs in the liver presumably occurs mainly in endosomes and lysosomes (7, 17, 18). We therefore tested the impact of a lag compartment before the fatty acids became available for oxidation or return to blood as FFAs. Such a delay compartment could be included but did not improve the model fit (supplementary Fig. V).

Tissue binding/uptake in whole body

The next step was to assemble a whole-body model including blood (Fig. 4). The sub-models for liver, adipose tissue, and heart were as derived above and the rate constants and remnants enter the liver with similar rate constants, in contrast to the marked difference noted for heart and adipose tissue where remnants showed much reduced binding affinity.

Hydrolysis of chylomicron TGs in the liver presumably occurs mainly in endosomes and lysosomes (7, 17, 18). We therefore tested the impact of a lag compartment before the fatty acids became available for oxidation or return to blood as FFAs. Such a delay compartment could be included but did not improve the model fit (supplementary Fig. V).

Tissue binding/uptake in other tissues

The time curves for label in the other tissues studied (spleen, kidney, lungs, and gastrocnemius muscle) were relatively flat and not informative for kinetic modeling.

Tissue binding/uptake in whole body

The next step was to assemble a whole-body model including blood (Fig. 4). The sub-models for liver, adipose tissue, and heart were as derived above and the rate constants

![Diagram](image-url)
were allowed to adjust (see legends to Figs. 4, 5 and Table 2 for details). Blood was modeled by two compartments, one with native chylomicrons and one consisting of lipolyzed chylomicrons that had returned from tissues (remnants). To simplify the fitting process, some of the rate constants were constrained as detailed in Fig. 4 and in Table 2. Scaling factors were included into the model to allow the chylomicron metabolism to be described as the sum of the metabolic events in liver-, adipose-, and heart-like tissues. Implicit in this is the assumption that other tissues behaved similar to liver, adipose tissue, or heart, or could be modeled as a mixture of these tissues. It was possible to simultaneously fit the experimental data for liver, heart, and adipose tissue in the fed and fasted states (Fig. 5 and Table 2). The process returned the following scaling factors: 1.05 ± 0.14 for liver-like tissues, and 15.8 ± 2.8/7.6 ± 0.6 (fed/fasted) for heart-like tissues, and 3.1 ± 7.4 g for adipose-like tissues.

The rate constants, derived in the whole-body model, predicted that the residence times for native chylomicrons in blood (compartment qB1/B3) were similar in the two nutritional states, 4.6 ± 2.1 min in fed and 4.1 ± 0.6 min in fasted rats. The main fate of the native chylomicrons was to bind reversibly in either heart-like or adipose-like tissues and return to the circulation as lipolyzed particles. This process occurred mainly in heart-like tissues, presumably representing chiefly muscles, and there was no difference with nutritional state in the fractions that went through this path (0.84 ± 0.07 in fed and 0.86 ± 0.02 in fasted rats). The model predicted that the particles stayed somewhat longer in heart-like tissues of fasted compared with fed rats, before the particles returned to the circulation (residence time 5.2 ± 0.6 min in fasted compared with 2.9 ± 1.4 min in fed rats). A lesser fraction of the chylomicrons that had bound and are being lipolyzed; qH1, qF1, and qL1 for core label and qH3, qF3, and qL3 for TG label. In heart an additional compartment, qH4, can be distinguished that contains chylomicrons/remnants that remain in the tissue but are lipolyzed much less rapidly than in qH3. The data for adipose tissue and liver does not contain enough information to distinguish analogous compartments. In all three tissues there is a storage compartment for TG label (qH5, qF5, and qL5). There is no need for storage of core label in heart or adipose tissue. There are two compartments in blood, qB1 for core, qB3 for TG label, into which the labeled chylomicrons were originally injected and another compartment (qB2 for core, qB4 for TG label) into which lipolyzed chylomicrons/remnants return from compartment qH2/qH4 in heart-like tissues and from compartment qF1/qF3 in adipose-like tissues. No chylomicrons/remnants return to blood from liver-like tissues.

**DISCUSSION**

In this study we have used kinetic modeling to explore the tissue binding and metabolism of chylomicron particles. For this we have used data on tissue radioactivity at
short times after injection of doubly labeled chylomicrons. A main conclusion is that when a chylomicron from the circulating blood has found an endothelial binding-lipolysis site, the particle remains at that site for one or several minutes. Models where chylomicron particles move rapidly between the circulating blood and endothelial sites cannot fit the data. This was most evident for heart of fasted rats. Core radioactivity, that traces the particles, rose gradually to a maximum at 7 min and then decreased rapidly. If the residence time for chylomicrons at endothelial binding sites was only a few seconds there would be an almost immediate peak, followed by a decay that paralleled

Fig. 5. Data fit for the integrated whole-body model. The panels show the experimental data points with their SEM for core (○, ○) and TG (■, □) label and the computer-derived model fits as solid lines. A, C, E, G: Show results for fed rats. B, D, F, H: Show results for fasted rats. A, B: Blood, sum of qB1+qB2 (core label) or qB3+qB4 (TG label). C, D: Liver, qL1 for core label, sum of qL3+qL5 for TG label. E, F: Adipose tissue, qF1 for core label, sum of qF3+qF5 for TG label. G, H: Heart, sum of qH1+qH2 for core label or qH3+qH4+qH5 for TG label. Adjustable parameters and constraints are shown in Table 2. Symbols as in Fig. 1.
TABLE 2. Constraints and adjustable parameters for full model

| Parameter Estimates |
|---------------------|
| Fed            | Fasted          |
| **Adjustable parameters** | **Fed** | **Fasted** |
| IC adipose-like tissue (%) | <0.01 | 0.22 ± 0.51 |
| IC heart-like tissue (%) | 22.8 ± 9.2 | 5.3 ± 3.6 |
| IC liver-like tissue (%) | 3.4 ± 0.9 | 2.2 ± 0.8 |
| $k_{f1,B1}$ (min$^{-1}$) | 0.012 ± 0.028 | 0.0029 ± 0.0071 |
| $k_{f2,F1}$ (min$^{-1}$) | 0.390 ± 0.06 | 0.16 ± 0.05 |
| $k_{f3,F3}$ (min$^{-1}$) | 1.2 ± 1.2 | 3.5 ± 0.27 |
| $k_{l1,L1}$ (min$^{-1}$) | 0.029 ± 0.011 | 0.032 ± 0.0007 |
| $k_{l1,B1}$ (min$^{-1}$) | 0.19 ± 0.13 | 0.24 ± 0.03 |
| $k_{l1,B2}$ (min$^{-1}$) | 0.10 ± 0.07 | 0.057 ± 0.010 |
| $k_{l2,F1}$ (min$^{-1}$) | 0.36 ± 0.17 | 0.20 ± 0.02 |
| $k_{l2,B1}$ (min$^{-1}$) | >10 | >10 |
| $k_{l2,B2}$ (min$^{-1}$) | 0.019 ± 0.004 | 0.015 ± 0.002 |
| $k_{l3,B1}$ (min$^{-1}$) | 6.0 ± 2.8 | 7.7 ± 0.9 |
| $k_{l3,B3}$ (min$^{-1}$) | 0.0047 ± 0.0059 | 0.014 ± 0.0007 |
| $k_{l3,L3}$ (min$^{-1}$) | 0.039 ± 0.004 | 0.040 ± 0.003 |
| $k_{l3,L5}$ (min$^{-1}$) | 0.48 ± 0.51 | 0.070 ± 0.026 |
| SF adipose-like tissue (g) | 3.1 ± 7.4 | 3.1 ± 7.4 |
| SF heart-like tissue (organs) | 15.8 ± 2.8 | 7.6 ± 0.6 |
| SF liver-like tissue (organs) | 1.05 ± 0.14 | 1.05 ± 0.14 |
| **Derived parameters** | **Fed** | **Fasted** |
| Residence time qB1 (min) | 4.6 ± 2.2 | 4.1 ± 0.6 |
| Residence time qB2 (min) | 6.5 ± 2.5 | 10.0 ± 1.0 |
| Residence time qF1 (min) | 3.4 ± 0.7 | 6.3 ± 2.0 |
| Residence time heart, core label (min) | 2.9 ± 1.4 | 5.2 ± 0.6 |

The model in Fig. 4 was fitted to data from Table 1 generating the data fits shown in Fig. 5. During the modeling process scaling factors for the three types of tissues (liver-like, heart-like, and adipose-like) were allowed to adjust to account for the metabolism of all the injected material in the entire body. Parameter estimates given as estimate ± SD from the modeling process; 95% confidence intervals can be calculated as estimate ± 2 × SD. Restrained parameters: $k_{f1,B1} = k_{F1,B1}, k_{l1,B1} = k_{L1,B1}, k_{F1,B3} = k_{F1,L3}, k_{l1,B3} = k_{L1,L3}, k_{l1,B5} = k_{l1,L5}, k_{l1,B4} = k_{l1,L4}, k_{l1,B3} = k_{l1,L3}, k_{l1,B4} = k_{l1,L4}, k_{l1,B2} = k_{l1,B2}$. Restricted to be equal between fed and fasted states: SF adipose tissue, SF liver, IC, initial condition; SF, scaling factor.

that of core radioactivity in blood. Our conclusion that chylomicrons reside for several minutes at endothelial sites is in accord with the concept of margination, defined as the difference between the apparent distribution volume of labeled TG-rich lipoproteins/lipid emulsion particles and the true plasma volume (5, 6, 17, 19–21). Ross, Pasatiempo, and Green (18) studied the metabolism of retinol-labeled chylomicrons (mainly in ester form) in lactating rats, that have high LPL activity, and compared the binding of chylomicrons in mammary glands of lactating rats, that have high LPL activity, and 1 day after the litter had been removed, when LPL has dropped to a much lower level (19). In the lactating rats 26%, but in the postlactating rats only 5% of retinol-labeled chylomicrons were in the mammary glands 2 min after injection.

On visual inspection, the time curves for radioactivity in heart and adipose tissue were different. So were time curves for fed compared with fasted animals, and for radioactivity in other tissues. All this data could, however, be fitted by the same basic model and the differences could be explained by differences in the rate constants rather than by differences in the model as such. A common feature was that the residence time of chylomicrons bound in the tissue was in the order of minutes, not seconds. A major difference was in the fate of the fatty acids released from chylomicron TGs. In the heart the fatty acid label was lost from the tissue. To what extent this occurred by oxidation or by return to blood as FFAs cannot be resolved from the present data. In adipose tissue of fed rats, the fatty acids were retained, presumably through resynthesis and storage of TGs. In the fasted state, the fatty acids were lost from the adipose tissue, presumably mainly through release to plasma FFAs. Net release of fatty acids from lipoprotein TGs in adipose tissue has been directly demonstrated by Frayn et al. (25).

To fit the data for core radioactivity in the heart, we had to allow for two types of chylomicron particles in blood. One, “native chylomicrons” were the injected material and had a high on-rate (“affinity”) for binding in heart and adipose tissue. Two, “lipolyzed chylomicrons” were formed from the native chylomicrons and had a lower on-rate for binding in heart and adipose tissue. This is in concert with the demonstration that when chylomicrons were injected to functionally eviscerated rats TG-depleted chylomicron particles (remnants) were formed and remained in the circulation (10, 26). The only tissue that effectively binds and catabolizes these particles is the liver (2, 27).

Uptake of chylomicrons/remnants by the liver is a complex process that involves a series of steps (2, 4, 7, 28). The particles have to pass through the fenestrae of the endothelium to enter the spaces of Disse. This adds a size-dependent parameter to the process (29). Then, the particles bind to the membranes of cells in the liver, through heparan-sulfate proteoglycans and/or specific receptors (30). These processes are accelerated if the particle has LPL attached to it, and when the particle acquires more apoE and/or when it interacts with hepatic lipase in the liver. The apparent affinity for sequestration in liver remained the same for chylomicron particles throughout the experiment, with no difference between native chylomicrons and lipolyzed chylomicrons. There was no sign of any lag time, as would be expected if the particles had to go through at least one round of lipolysis in peripheral tissues before they could be taken up in the liver (11). We tried to force such a delay in the model but this worsened, rather than improved, the fit to the experimental data.

The peak of core radioactivity was higher in hearts of fasted compared with fed rats. Modeling returned a longer residence time for chylomicrons in hearts of fasted than fed rats. This would be expected if the binding of chylomicrons in heart were mediated by, or at least mainly by, the lipase itself. There is much evidence that in heart, functional LPL is higher in the fasted state (15, 22–24). Strong evidence for the hypothesis that LPL itself is a main ligand for binding of chylomicrons in tissues comes from the recent study by Ross, Pasatiempo, and Green (18). They compared the binding of chylomicrons in mammary glands of lactating rats, that have high LPL activity, and 1 day after the litter had been removed, when LPL has dropped to a much lower level (19). In the lactating rats 26%, but in the postlactating rats only 5% of retinol-labeled chylomicrons were in the mammary glands 2 min after injection.

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Actually, this is in agreement with a previously published model by Redgrave and Zech (31), where chylomicron remnants had almost the same rate of uptake in the liver as less heavily lipolyzed chylomicrons.

We cannot resolve the details of the hepatic sequestration/binding/catabolism of native and lipolyzed chylomicrons. Our data only show the sum of all these processes. What we can say is that the processes start immediately after the chylomicrons enter the circulation and then continue with no apparent change in overall rate constant. Furthermore, the data indicate that few, if any, of the particles that have been sequestered in the liver return to the circulating blood, in sharp contrast to the case for heart and adipose tissue.

The disappearance of chylomicrons or chylomicron-like emulsions from blood has usually been fitted to single exponentials, a more rapid one for TG label and a slower one for core label (6, 31, 32). Our previously published model on chylomicron metabolism showed loss of TG label by lipolysis in the blood compartment and not from tissue compartments (6). The process we describe here, where chylomicrons bind and remain in tissues for several minutes and then return to the circulating blood, should give a more complex curve. The blood curves generated in the whole-body model do not, however, deviate much from that for single exponentials. A large fraction of the chylomicrons enter the liver by a single exponential process. The sum of native and lipolyzed chylomicrons in the margined pools in other tissues is 38% at most. There is gradual entrance of particles into and return from these pools (supplementary Fig VI). Taken together in the whole-body model, these processes generate disappearance curves that do not deviate much from single exponentials, which explains why they have escaped notice in previous studies.

The exact nature of margination, how chylomicrons, native and remnants, bind to endothelium for lipolysis remains to be elucidated (33). One potential mechanism might be that chylomicrons bind to heparan-sulfate proteoglycans before interaction with LPL (34, 35). A second is that chylomicrons bind to LPL, which in turn depends on glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1 (GPIHBP1) for localization to the endothelial surface. A third is that chylomicrons interact directly with GPIHBP1 (33). Future studies using chylomicrons, blocking agents, genetic variants, and kinetic modeling might shed further light on this intriguing question.

REFERENCES

1. Abumrad, N. A., and N. O. Davidson. 2012. Role of the gut in lipid homeostasis. Physiol. Rev. 92: 1061–1085.
2. Redgrave, T. G. 2004. Chylomicron metabolism. Biochem. Soc. Trans. 32: 79–82.
3. Havel, R. J. 1998. Receptor and non-receptor mediated uptake of chylomicron remnants by the liver. Atherosclerosis. 141(Suppl 1): S1–S7.
4. Yu, K. C., and A. D. Cooper. 2001. Postprandial lipoproteins and atherosclerosis. Front. Biosci. 6: D332–D354.
5. Karpe, F., T. Olivecrona, A. Hamsten, and M. Hultin. 1997. Chylomicron/chylomicron remnant turnover in humans: evidence for margination of chylomicrons and poor conversion of larger to smaller chylomicron remnants. J. Lipid Res. 38: 949–961.
6. Hultin, M., R. Savonen, and T. Olivecrona. 1996. Chylomicron metabolism in rats: lipolysis, recirculation of triglyceride-derived fatty acids in plasma FFA, and fate of core lipids as analyzed by compartmental modelling. J. Lipid Res. 37: 1022–1036.
7. Havel, R. J., and R. L. Hamilton. 1988. Hepatic lipoprotein receptors and intracellular lipoprotein catabolism. Hepatology. 8: 1040–1074.
8. Foster, D. M. 1998. Developing and testing integrated multicompartment models to describe a single-input multiple-output study using the SAAM II software system. Adv. Exp. Med. Biol. 445: 59–78.
9. Jacquez, J. A. 1996. Compartmental Analysis in Biology and Medicine. 3rd edition. University of Michigan Press, Ann Arbor.
10. Redgrave, T. G. 1970. Formation of cholesteryl ester–rich particulate lipid during metabolism of chylomicrons. J. Clin. Invest. 49: 465–471.
11. Teusink, B., P. J. Voshol, E. V. Dahlmans, P. C. Rensen, H. Pijl, J. A. Romijn, and L. M. Havekes. 2003. Contribution of fatty acids released from lipolysis of plasma triglycerides to total plasma fatty acid flux and tissue-specific fatty acid uptake. Diabetes. 52: 614–620.
12. Felts, J. M. 1965. The metabolism of chylomicron triglyceride fatty acids by perfused rat livers and by intact rats. Ann. N. Y. Acad. Sci. 131: 24–33.
13. Olivecrona, G. O. T. 2009. The ins and outs of adipose tissue. In Cellular Lipid Metabolism. C. Ehnholm, editor. Springer Verlag, Hidelberg, 315–369.
14. Eaton, R. P., M. Berman, and D. Steinberg. 1969. Kinetic studies of plasma free fatty acid and triglyceride metabolism in man. J. Clin. Invest. 48: 1560–1579.
15. Borensztajn, J., and D. S. Robinson. 1970. The effect of fasting on the utilization of chylomicron triglyceride fatty acids in relation to clearing factor lipase (lipoprotein lipase) releasable by heparin in the perfused rat heart. J. Lipid Res. 11: 111–117.
16. Nilsson-Ehle, P., A. S. Garfinkel, and M. C. Schotz. 1980. Lipolytic enzymes and plasma lipoprotein metabolism. Annu. Rev. Biochem. 49: 667–693.
17. Jakke, S., E. Runquist, S. Brady, R. L. Hamilton, and R. J. Havel. 1991. Isolation and characterization of three endosomal fractions from the liver of normal rats after lipoprotein loading. J. Lipid Res. 32: 486–498.
18. Ross, A. C., A. M. Pasatempo, and M. H. Green. 2004. Chylomicron margination, lipolysis, and vitamin A uptake in the lactating rat mammary gland: implications for milk retinoid content. Exp. Biol. Med. (Maywood). 229: 46–55.
19. Hamosh, M., T. R. Clary, S. S. Chernick, and R. O. Scow. 1970. Lipoprotein lipase activity of adipose and mammary tissue and plasma triglyceride in pregnant and lactating rats. Biochem. Biophys. Acta. 210: 473–482.
20. Harris, K. L., and P. A. Harris. 1973. Kinetics of chylomicron triglyceride removal from plasma in rats: effect of dose on the volume of distribution. Biochem. Biophys. Acta. 326: 12–16.
21. Redlinhoef, A. F., M. J. Malloy, J. P. Kane, and R. J. Havel. 1986. Metabolism of apolipoproteins B-48 and B-100 of triglyceride-rich lipoproteins in patients with familial dysbetalipoproteinemia. J. Clin. Invest. 78: 722–728.
22. Pedersen, M. E., and M. C. Schotz. 1980. Rapid changes in rat heart lipoprotein lipase activity after feeding carbohydrate. J. Nutr. 110: 481–487.
23. Pulinkunnil, T., A. Abrahani, J. Varghese, N. Chan, I. Tang, S. Ghosh, J. Kulpa, M. Allard, R. Brownsey, and B. Rodrigues. 2003. Evidence for rapid “metabolic switching” through lipoprotein lipase occupation of endothelial-binding sites. J. Mol. Cell. Cardiol. 35: 1093–1103.
24. Wu, G. L., Zhang, J. Gupta, G. Olivecrona, and T. Olivecrona. 2007. A transcription-dependent mechanism, akin to that in adipose tissue, modulates lipoprotein lipase activity in rat heart. Am. J. Physiol. Endocrinol. Metab. 293: E908–E915.
25. Frayn, K. N., S. Shadid, R. Hamlani, S. M. Humphreys, M. L. Clark, B. A. Fielding, O. Boland, and S. W. Coppack. 1994. Regulation of fatty acid movement in human adipose tissue, modulates lipoprotein lipase activity in rat heart. J. Lipid Res. 35: 111–117.
26. Frain, K. N., S. Shadid, R. Hamlani, S. M. Humphreys, M. L. Clark, B. A. Fielding, O. Boland, and S. W. Coppack. 1994. Regulation of fatty acid movement in human adipose tissue, modulates lipoprotein lipase activity in rat heart. J. Lipid Res. 35: 111–117.
27. Cooper, A. D. 1997. Hepatic uptake of chylomicron remnants. J. Lipid Res. 38: 2173–2192.
28. Heeren, J., T. Grewal, S. Jackle, and U. Beisiegel. 2001. Recycling of apolipoprotein E and lipoprotein lipase through endosomal compartments in vivo. J. Biol. Chem. 276: 42353–42338.
29. Fraser, R., B. R. Dobbs, and G. W. Rogers. 1995. Lipoproteins and the liver sieve: the role of the fenestrated sinusoidal endothelium in lipoprotein metabolism, atherosclerosis, and cirrhosis. Hepatology. 21: 863–874.
30. Mahley, R. W., and Z. S. Ji. 1999. Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E. J. Lipid Res. 40: 1–16.
31. Redgrave, T. G., and L. A. Zech. 1987. A kinetic model of chylomicron core lipid metabolism in rats: the effect of a single meal. J. Lipid Res. 28: 473–482.
32. Borén, J., M. R. Taskinen, and M. Adiels. 2012. Kinetic studies to investigate lipoprotein metabolism. J. Intern. Med. 271: 166–173.
33. Young, S. G., and R. Zechner. 2013. Biochemistry and pathophysiology of intravascular and intracellular lipolysis. Genes Dev. 27: 459–484.
34. Merkel, M., R. H. Eckel, and I. J. Goldberg. 2002. Lipoprotein lipase: genetics, lipid uptake, and regulation. J. Lipid Res. 43: 1997–2006.
35. Cryer, A. 1989. The role of the endothelium in myocardial lipoprotein dynamics. Mol. Cell. Biochem. 88: 7–15.