Chylomicron metabolism in rats: lipolysis, recirculation of triglyceride-derived fatty acids in plasma FFA, and fate of core lipids as analyzed by compartmental modelling

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Abstract Chylomicrons labeled in vivo with [14C]oleic acid (primarily in triglycerides (TG), providing a tracer for lipolysis) and [3H]retinol (primarily in ester form, providing a tracer for the core lipids) were injected into rats. Disappearance of the two labels from plasma and appearance of label in plasma free fatty acids (FFA) were analyzed by compartmental modelling. Both core and TG label distributed into an apparent volume 10–15% larger than the blood volume. Part of this probably represents margination to endothelial-binding-lipolysis sites. An open two-compartmental model for plasma FFA was derived from experiments where unesterified oleic acid complexed to albumin was injected. Applying this model revealed that most of the oleic acid from chylomicron triglycerides mixes with the FFA. The disappearance of chylomicron core label required a model in which the label transfers into a second compartment before it leaves the blood. The rate constant for the transformation was high and predicted that, on average, chylomicron spent less than 2 min in the first compartment. The rate out from the second compartment predicted that about 60% of the core label left blood while, on average, chylomicron retained more than half of its triglyceride molecules, i.e., after rather limited lipolysis. The mechanism by which the core label leaves blood is not clear. Modelling showed that under the assumption that the process is shared by chylomicron triglycerides, about half of them go out by this pathway. Comparing fed and fasted rats, the main differences were in the turnover of FFA and in the extent to which chylomicron TG label reappeared in the FFA. ■ This study indicates that a large fraction of the triglycerides in chylomicrons leave plasma together with the core lipids and that most of the fatty acids from chylomicron triglycerides mix into the same metabolic compartments as do plasma free fatty acids.—Hultin, M., R. Savonen, and T. Olivecrona. 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.* 1996. **37:** 1022–1036.

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Chylomicrons carry dietary lipids into the blood stream. There, they interact with lipoprotein lipase at the vascular endothelium in extrahepatic tissues, which results in progressive removal of triglycerides through hydrolysis. During this process core lipids such as retinyl and cholesteryl esters are thought to remain with the particle as it is transformed into a remnant. In the rat, these remnants are believed to be almost quantitatively taken up by receptor-mediated endocytosis in the liver. We have recently been involved in studies on chylomicron metabolism in rats after heparin administration (1), in comparison to a lipid emulsion (2, 3), and during an experimental triglyceride-clamp (4). During these studies it became evident that it would be useful to have a tool for a more thorough analysis of the data. Qualitatively, the results illustrate that triglycerides are removed more rapidly than the core lipids, that this occurs through hydrolysis, and that some of the fatty acids generated in the hydrolysis step appear in the plasma free fatty acids (FFA) pool. It is, however, not possible to estimate the quantitative aspects of these pathways directly from the data. To extract this information we turned to compartmental modelling.

Some of the questions we wanted to approach were: how much of the chylomicron triglycerides are removed by lipolysis before the particle goes out as a remnant? What fraction of the released fatty acids return to blood as albumin-bound FFA and what fraction goes directly to tissue metabolism? Many studies have been done on lipoprotein metabolism (see refs. 5, 6 for review) but only in a few studies has compartmental modelling been used to analyze chylomicron metabolism (7–10). Berr (11) showed that chylomicrons labeled with retinyl

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palmitate were cleared in a biexponential manner. Green, Massaro, and Green (8) used a delipidation cascade to model the clearance of cholesterol labeled chylomicrons. Redgrave and Zech (9) used a complex model including a delipidation chain, a remnant compartment, and a model for the recirculation of labeled lipids in the liver to study TG and cholesteryl ester label contained in chylomicrons. Recently, Redgrave et al. (10) used compartmental modelling to calculate residence times for core and triglyceride label contained in chylomicrons and in lipid emulsions. This model had two compartments for the chylomicrons in blood, uptake for recirculation from the first and removal from the other. A major drawback with these compartmental models (7–10) was that the appearance of labeled FFA in blood from lipolysis of chylomicron triglycerides was not included.

Some basic questions became apparent as we started our work. Is it appropriate to use isolated chylomicrons, or should whole lymph be used? Are retinyl esters an appropriate tracer for core lipids or are retinyl esters themselves hydrolyzed by lipoprotein lipase (12)? Is the distribution volume for chylomicrons different from the blood volume?

When these questions had been addressed we proceeded to generate data on the disappearance from blood of chylomicron triglycerides and core lipids and to analyze these data by some simple compartmental models. As a complement we have studied the tissue distribution of the labeled lipids. We have also used the model to analyze some earlier data on the metabolism of lipid emulsions (3).

MATERIALS AND METHODS

Preparation of tracer labeled materials

Lymph chylomicrons were obtained according to Bollman, Cain, and Grindlay (13) using rats weighing 250–300 g. Briefly, one thin plastic tubing was placed in the thoracic duct and a second in the stomach. After the operation, the rats were kept in restraining cages. A solution containing 5% glucose, 0.85% NaCl, and 0.05% KCl was infused through the stomach tubing at a rate of 2.5 ml/h. In addition, the rats had access to drink the same solution. Lymph flow was 2–3 ml/h during the day and higher during the night. The rats were allowed to recover overnight before the preparation of labeled chylomicrons began.

Two species of labeled chylomicrons were prepared. At 8 AM the morning after the implantation of the tubing, rats were given 2 ml 10% Intralipid® (Pharmacia Hospital Care, Stockholm, Sweden) into which either 150 µCi[^{3}H]retinol and 100 µCi[^{14}C]oleic acid or 100 µCi[^{3}H]oleic acid had been incorporated by sonication. White lymph appeared in the cannula after about 0.5 h and was collected into sterile tubes at room temperature. EDTA and gentamicin were added to give final concentrations of 0.5 and 0.1 mg per ml, respectively. The lymph was defibrinated and the white blood cells were removed by a brief centrifugation in a table-top centrifuge. In some experiments the lymph was used as such. To isolate chylomicrons the lymph was layered under 0.9% NaCl, 0.05% EDTA, 0.01% gentamicin and the tubes were centrifuged for 30 min at 35,000 rpm in a Beckman Sw 50 rotor at 4°C. The isolated chylomicrons were resuspended in the same buffer to a final concentration of 20 mg triglycerides per ml, i.e., 4 mg triglycerides per 0.20 ml solution, stored under nitrogen, and used within 48 h. More than 97% of the labeled oleic acid was present as TG, as determined by TLC, and the remainder mainly as phospholipids.

To prepare a tracer for plasma FFA, labeled (^{3}H or ^{14}C) oleic acid was dried down on the walls of a test tube, rat citrate plasma was added, and the tube was gently agitated in a water-bath at 37°C for 1 h. Throughout this, the sample was kept under nitrogen. The preparation was then mixed with chylomicrons to a final concentration of 20 mg triglycerides and 0.5 ml plasma per ml.

Red blood cells (RBC) were labeled by allowing Na$_2$^{51}CrO$_4$ to incorporate into isolated rat RBC for 2 h at 37°C (14). The RBC were washed four times by resuspension in saline. After the final centrifugation less than 0.5% of the radioactivity was found in the supernatant. The radioactivity in 0.2 ml of the final RBC suspension corresponded to about 500,000 cpm.

All radiochemicals were obtained from NEN-DuPont, Stockholm, Sweden.

Animal procedures

Male Sprague-Dawley rats were obtained from Moellegard Breeding Centre, Skensved, Denmark. They were housed in a 12-h light/12-h dark cycle and had free access to standard pellet diet and water. The rats were allowed to recover from transportation for at least 10 days before the experiments. For experiments with fasted rats, the food was removed from the cages at noon the day before the experiment. All experiments were carried out between 8 and 12 AM. The rats were anesthetized by an intramuscular injection of Hypnorm (0.5 ml/kg, AB Leo, Helsingborg, Sweden, 0.315 mg fentanyl citrate/ml and 10 mg fluanisone/ml) and Stesolid Novum (0.5 ml/kg, 5 mg diazepam/ml). All animal procedures were approved by the Animal Ethics Committee of Northern Sweden. Blood volume was calculated as 5.5% of the body weight (14).

For the experiments with labeled lipids, anesthetized rats weighing 180–210 g were given 0.20 ml of the

labeled material in an exposed jugular vein. In the case of chylomicrons this corresponded to 4 mg triglycerides and between 0.25 and 1 µCi of each label. In each experiment the injected radioactivity was determined using the same syringe to inject directly into scintillation vials and into tubes for further processing along with the blood samples. Serial blood samples, approximately 0.20 ml each, were taken and put into preweighed tubes. After 20 min the rats were killed by exsanguination through the abdominal aorta. Tissues were dissected out, rinsed in saline, blotted dry, weighed, and frozen. Later they were thawed and lipids were extracted as described below.

To determine the apparent volume of distribution for the chylomicrons in relation to the blood volume, rats were anesthetized and 0.20 ml of the labeled RBC suspension was injected followed 1 min later by 0.20 ml chylomicrons. Blood samples were taken after 1, 3, 7, 11, and 15 min. From each sample about 0.20 ml was used to extract radioactive lipids as described below and 0.15 ml for gamma-counting in an LKB 1480 Wizard. In both cases the sample was taken into preweighed tubes and the exact amount of blood was determined by weight.

Lipid extraction

The blood samples were transferred within 7 sec into tubes containing 2 ml isopropanol–heptane–1 M H_2SO_4 40:10:1 (15). The tubes were carefully vortexed before and after the addition of 1.5 ml heptane and 1.2 ml distilled water. One ml of the upper phase was taken to new tubes and 1 ml 50 mM NaOH in 50% ethanol–water (v:v). After mixing and centrifugation, an aliquot was taken from the upper phase for determination of radioactivity in triglycerides and retinyl esters. The lower phase was washed two times by addition of 3 ml heptane, vortexing and centrifugation, before a sample was taken for determination of radioactivity in unesterified fatty acids. Four ml of scintillation liquid (OptiPhase Hisafe III, Pharmacia, Uppsala, Sweden) was added to each sample which was then counted in a LKB-Pharmacia 1214 β-counter.

In most experiments tissues were homogenized using a Polytron (Kinematica, Basel, Switzerland) and extracted in chloroform–methanol 2:1 (v:v, 30 ml per g wet weight). An aliquot of the extract was centrifuged for 10 min to remove tissue residues. Three ml of the clear phase was added to 1.2 ml 2% KH_2PO_4 in water and shaken. After an additional centrifugation to separate the two phases, the upper phase was discarded and 1 ml of the lower phase was dried down in a scintillation vial for determination of radioactivity.

For the experiment in Table 4 the rats were cut into about 140 small pieces, either approx. 1 g each, or

following anatomical borders. The pieces were dissolved overnight in 6 M KOH in 50% ethanol (1 ml per gram tissue) at room temperature (16). For the skeleton a modified procedure was used. The bones were first carefully cleaned from muscles and then separated into upper limb, lower limb, head, and torso. Then remaining soft tissue was removed by treatment with the ethanolic KOH solution for 30 min at 37°C. At this time the bones remained intact. They were fully dissolved by continued treatment with ethanolic KOH overnight. This dissolved the bones and made it possible to extract the lipids trapped inside the bones. Heptane was added to each sample which was then acidified with HCl using thymol blue as indicator. Aliquots of the upper phase containing lipids and fatty acids were taken, scintillation cocktail was added, and the radioactivity was determined.

For determination of the whole body recovery of labeled lipids, the rats were saponified in a mixture of 300 ml ethanol, 100 ml water, and 100 g KOH (3). The lipids were extracted, as previously described (3), and the radioactivity associated with the extracted lipids was determined.

In vitro lipolysis and separation of lipolysis products

Chylomicrons (1 mg triglycerides/ml) were incubated in vitro with purified bovine lipoprotein lipase (17) in 0.15 M Tris-Cl, pH 8.5, 10% BSA, 0.1 M NaCl, and 10 µg heparin (Löwens, Malmö, Sweden) per ml in a total volume of 200 µl. The reaction was stopped by addition of 3 ml chloroform–methanol 2:1. Lipolysis products were separated by thin-layer chromatography using activated Silica-G and heptane–diethyl ether–acetic acid 80:20:1 as liquid phase (18). The spots corresponding to triglycerides, retinyl esters, fatty acids, and retinol were scraped off and counted in a β-counter.

Compartmental modelling and statistical procedures

For the modelling (19) we used the SAAM II program from the SAAM Institute, University of Washington, Seattle, WA, on a SUN SparcClassic workstation. The process was divided into two steps. First, the general model was developed. For this, we used the mean values for the experiments (five or more rats) to minimize the influence of outliers. Then, data for each individual rat were fitted to the model, one at a time. At this stage obvious outliers in the data were excluded from the fitting. This resulted in five or more sets of values for the model parameters for each group of rats. In some cases (Tables 1 and 3) means and SEM for these sets of parameters are given.

In most experiments groups of fed and fasted rats were studied. The primary objective was that the model should be able to fit data in both nutritional states, as a

test of generality. As the next step we tested what parameters could be restrained to be the same in both nutritional states without substantially worsening the fit, and what parameters had to be allowed to differ. For this, the parameters were simultaneously fitted to the mean values for fed and fasted rats.

ANOVA analysis or Kruskal-Wallis test was used to test for statistical significance using the SPSS for Windows program (version 6.1, SPSS Inc., Chicago, IL). Data presented are means ± SEM, unless otherwise specified.

RESULTS

Chylomicron preparation used

To explore whether the isolation procedure used here changes the metabolic behavior of the chylomicrons, we compared isolated chylomicrons to whole chyle. **Figure 1** shows the clearance from blood. Both the core label ([³H]retinyl esters) and the TG label ([¹⁴C]oleic acid in triglycerides) showed the same qualitative kinetics for whole chyle and isolated chylomicrons. As observed previously by several investigators, the TG label disappeared more rapidly than the core label (1, 20–22). Thirteen minutes after injection of whole lymph and isolated chylomicrons, respectively, 24.4 ± 2.2% and 25.4 ± 0.9% of the core label, and 7.9 ± 1.5% and 7.0 ± 1.4% of the TG label remained in blood. Hence the clearance rates were similar. Radioactive fatty acids appeared in the plasma FFA. At 13 min, 0.92 ± 0.09% and 0.87 ± 0.07% of the labeled oleic acid was in FFA after injection

Fig. 2. Comparison of the rates at which retinyl esters and triglycerides in chylomicrons are hydrolyzed by lipoprotein lipase (LPL). Doubly labeled chylomicrons were incubated with the enzyme and the products were analyzed by thin-layer chromatography as described in Methods. Hydrolysis of retinyl esters to retinol (□, ■) and of triglycerides to fatty acids (○, ●). Main graph, open symbols, 50 mU lipoprotein lipase per ml incubation mixture. Inset, closed symbols, 4000 mU lipoprotein lipase per ml incubation mixture. Results are expressed as percent of radioactivity present in retinol or fatty acids, respectively.

of whole lymph and isolated chylomicrons, respectively. The tissue distribution of label was also similar. Twenty minutes after injection, the liver contained 59.9 ± 2.4% of the core label and 18.1 ± 0.08% of the TG label from whole lymph. From isolated chylomicrons the values were 63.8 ± 3.4% and 19.2 ± 0.8%, respectively. The spleen contained 2.74 ± 0.37% of the core label and 1.00 ± 0.17% of the TG label from whole lymph, and 3.15 ± 0.34% and 1.12 ± 0.09% from chylomicrons, respectively. The carcass was saponified and the lipid soluble radioactivity was determined. Overall recovery of radioactive oleic acid was 59% from whole lymph and 62% from chylomicrons. Hence, about 40% of the oleic acid had been oxidized, i.e., converted to water-soluble products or CO_2. For none of these values was the difference between isolated chylomicrons and whole lymph statistically significant.

From these results we concluded that chylomicrons isolated by the methods used here provide an appropriate tracer for lipid transport after a fat-rich meal.

Retinyl ester hydrolysis

A basic assumption was that the retinyl esters provide a label for the core lipids. This presupposes that retinyl esters are not hydrolyzed or transferred to other lipoproteins before the chylomicrons/remnants are removed from the circulation. It was, however, recently reported that retinyl esters are hydrolyzed by lipoprotein lipase (12). To test the extent of such hydrolysis with the chylomicrons used here, we incubated them in vitro with lipoprotein lipase (**Fig. 2**). In the first experiments
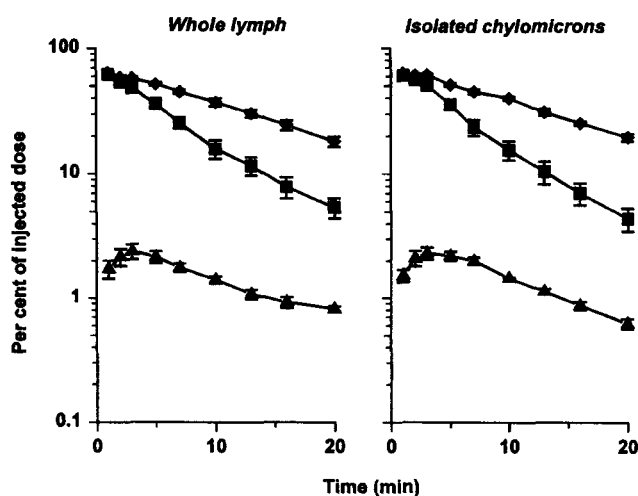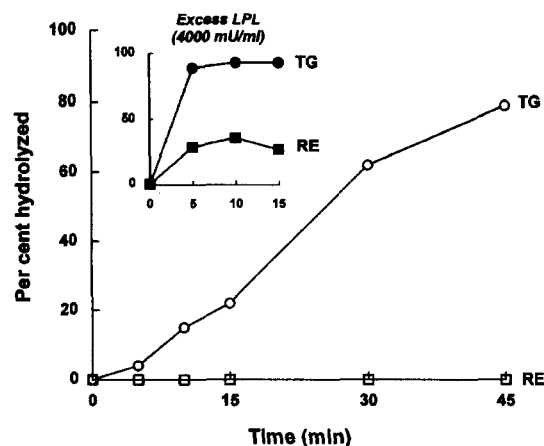

Fig. 1. Comparison of the clearance of core and TG label from whole lymph and from isolated chylomicrons. Rats were fasted overnight, anesthetized, and given an i.v. injection of 4 mg triglycerides either as whole lymph (left panel) or as chylomicrons isolated from the lymph (right panel). Core label (◆), TG label (■), and reappearance of TG label as FFA (▲). Data are mean ± SEM (n = 5).

we added moderate amounts of the enzyme so that about 25% of the triglycerides were hydrolyzed in 15 min. Under these conditions there was no detectable hydrolysis of retinyl esters. Even after 45 min we could not detect any increase in free retinol. At this time more than 70% of the triglycerides had been hydrolyzed. We then repeated the experiment with 80 times more lipase, corresponding to the amount used by Blaner et al. (12). Now, more than 90% of the triglycerides were hydrolyzed within 5 min, and there was substantial hydrolysis also of the retinyl esters, about 30%. These experiments show that with a large excess of the lipase some retinyl esters are hydrolyzed, but this is probably not quantitatively important during the metabolism of chylomicrons under physiological conditions.

Apparent distribution volume of injected chylomicrons

Because to determine the flux of material it is necessary to have an estimate of the initial apparent volume of distribution, i.e., that volume in which the injected tracer material distributes instantaneously and uniformly, and as this is often taken to be the plasma volume, labeled red blood cells (RBC) were injected together with labeled chylomicrons. The labeled RBC permitted an estimate of blood volume that could be compared with the estimated initial volume of distribution of chylomicrons using the standard dilution technique, i.e., extrapolation to time zero knowing the initial dose.

Previous studies in our laboratory indicated that the blood volume of the type of rats used here is about 5.6% of body weight (14). With this figure, extrapolation of the disappearance curves in Fig. 1 gave intercepts of only about 80% for both core and TG label, suggesting that the chylomicrons distributed into a larger volume. To study this more directly, ^{51}Cr-labeled red blood cells (RBC) were injected 1 min before the chylomicrons (**Fig. 3**). By fitting the data as a monoexponential decay, the apparent distribution volumes were calculated. For RBC the distribution volume was 11.3 ± 0.3 ml in fasted and 12.4 ± 0.3 ml in the fed rats, which corresponds to 5.6% and 5.6% of body weight, respectively. This is similar to the values we have observed before (14). The apparent distribution volumes for the chylomicron labels were significantly larger than for RBC, 12.7 ± 0.9 and 13.3 ± 0.4 ml for the core label and 13.5 ± 1.0 and 13.9 ± 0.3 ml for the TG label in fasted and fed rats, respectively. There were no statistically significant differences between the volumes estimated for the core and TG labels or between the nutritional states, but all of these values were significantly larger than for RBC ($P < 0.01$). These data show that chylomicrons distribute into a larger apparent volume than the circulating blood, i.e., that

some of the chylomicrons are not seen in blood. In the following we will refer to this distribution factor as the fraction in blood (FIB), which in these cases was 0.90 and 0.93 for core label, and 0.85 and 0.89 for TG label in fasted and fed rats, respectively.

FFA turnover

To model the recirculation of chylomicron-derived fatty acids it was necessary to have a model for the turnover of plasma FFA. For this, labeled oleic acid was complexed to albumin and injected to fed rats. The label disappeared rapidly until only a few percent remained in blood and then more slowly (**Fig. 4**). Results were similar when ^{3}H- or ^{14}C-labeled oleic acid was used (data not shown).

The data could readily be fitted to the model proposed by Eaton, Berman, and Steinberg (23) (Fig. 4), where FFA (compartment 4) feed rapidly into another, much larger, pool (compartment 5) representing tissue lipids that exchange with the FFA, and there is a pathway for irreversible loss of material from the FFA pool. This represents oxidation and storage as lipid esters that do not feed back significantly into the FFA over the time of our experiments. To fit the data it was necessary to allow compartment 4 to have a larger volume than blood. This presumably represents FFA in membranes, tissue spaces, and in other extravascular spaces. It should be noted that the model was not intended to probe the metabolism of FFA, but was used as a "black box" to describe the behavior of label that entered plasma FFA from chylomicrons.

One possible source of error was that the labeled oleic acid had not formed appropriate complexes with albu-
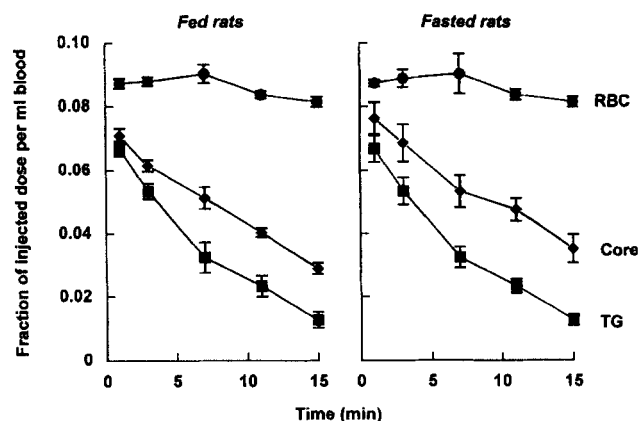

Fig. 3. Apparent distribution volume of doubly labeled chylomicrons compared to ^{51}Cr-labeled red blood cells. The red blood cells (RBC, ●) were injected 1 min before the chylomicrons (core label ◆, TG label ■) to anesthetized fed and fasted rats. Serial blood samples were taken, and the radioactivities were determined as described in Methods. The results are expressed as fraction of injected dose in 1 ml of blood. Mean ± SEM (n = 5).
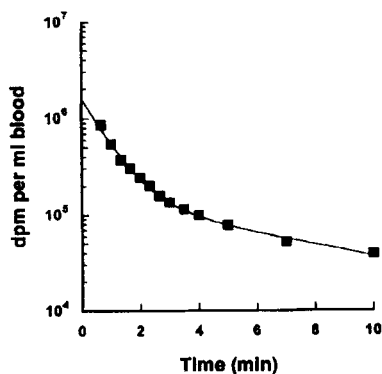
Fig. 4. Turnover of FFA. Albumin-bound [^{3}H]oleic acid was injected into an anesthetized, fed rat. Serial blood samples were taken and extracted as described in Methods. The data were fitted to the model in the lower panel. Compartment 4 represents plasma FFA and fatty acids that mix almost instantaneously with the plasma FFA. Compartment 5 represents tissue lipids that exchange with the plasma FFA. There is a pathway out from compartment 4 which represents oxidation and storage in lipids that do not feed back to the plasma FFA. (■) shows observed data for ^{3}H-FFA in a representative rat out of nine studied. The curve shows the model fit. The syringe (⌐) shows where the tracer was introduced. The open circle with the dotted line (...) indicates the compartment or connects compartments that correspond to the samples analyzed, and includes the fraction in blood (FIB).

min. To explore this, TG-labeled chylomicrons were incubated with rat postheparin plasma in the presence of excess albumin. Lipolysis was allowed to proceed for 1 h at 37°C. After 10 min the mixture had already cleared. At the end of the incubation the sample was ultracentrifuged to float the chylomicrons/chylomicron remnants. Aliquots of the bottom phase were then injected to rats and the clearance of labeled fatty acids was followed. The disappearance curves were similar to that in Fig. 4. Thus, fatty acids generated by lipolysis in a plasma-albumin medium behaved in the same way as the complexes used in our other experiments.

Simultaneous fitting of data for FFA and triglyceride turnover

Next, the turnover of chylomicron triglycerides and recirculation of chylomicron-derived fatty acids in the plasma FFA fraction was modelled (**Fig. 5**). The simplest model would be that turnover of triglycerides is solely by lipolysis and that the fatty acids mix with the FFA pool. This gives an open three-compartmental model with irreversible transfer of material from chylomicron triglycerides (compartment 1) into the FFA pool (com-

partment 4). To test this, we used data from an experiment where [^{3}H]oleic acid-labeled chylomicrons and ^{14}C-labeled unesterified oleic acid were injected together. All parameters in the FFA part of the model ($k_{5,4}$, $k_{4,5}$, $k_{0,4}$, and FIB) were constrained to be the same for fatty acids injected as the albumin complex and derived from chylomicrons.

This model predicts that plasma TG label decays following a single exponential, which agrees with the data (Fig. 5), except that in almost all rats the predicted value for TG label in blood was below the experimental value at the last point, 20 min. A possible reason was that labeled fatty acids from chylomicron triglycerides had been reesterified in the liver and transferred back to plasma in endogenous lipoproteins. To explore this we injected unesterified [^{14}C]oleic acid and followed radioactivity in lipid esters in plasma. Label started to appear between 10 and 13 min after injection (data not shown). This is similar to previous estimates for the time required for lipoprotein assembly and transport out from the liver (24–26). The amount of labeled fatty acids recovered in plasma lipid esters increased with time and reached $1.45 \pm 0.45\%$ and $0.74 \pm 0.07\%$ at 20 min in fed and fasted rats, respectively. The radioactivities in
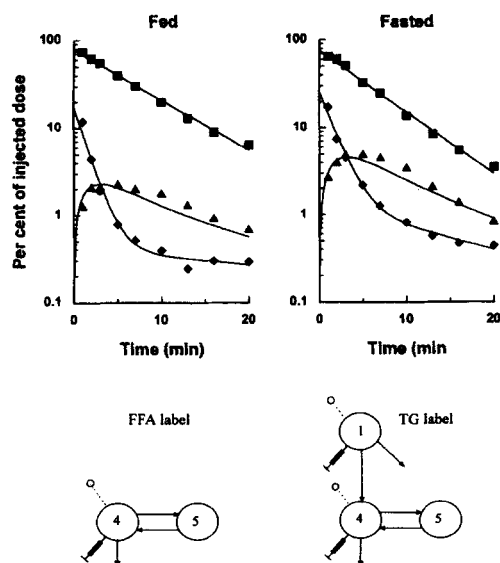

Fig. 5. Turnover of FFA and of chylomicron triglycerides. ^{14}C-labeled oleic acid complexed to albumin was mixed with [^{3}H]oleic acid-labeled chylomicrons and injected to anesthetized rats. The data were fitted to the model shown in the lower panel. Compartments 4 and 5 are the same as in Fig. 4. Compartment 1 represents chylomicron triglycerides. There is a pathway out from this compartment that represents transfer of label to tissue metabolism without mixing with the FFA pool. The left and right upper panels show observed data for ^{3}H-FFA (▲), ^{3}H-TG (■), and ^{14}C-FFA (◆) in a representative fed and fasted rat, respectively, from groups of five. The curves are the derived fits. Model symbols as in Fig. 4.

TABLE 1. Model parameters for the experiment in Fig. 5

		Fed	Fasted	Statistics
FIB (chylomicron TG)	fraction in blood	0.83 ± 0.02	0.85 ± 0.02	n.s.
FIB (free fatty acids)	fraction in blood	0.21 ± 0.02	0.25 ± 0.02	n.s.
FCR (triglycerides)	min^{-1}	0.12 ± 0.03	0.15 ± 0.01	n.s.
TG label to comp 4	fraction	0.81 ± 0.08	1.00	$P < 0.06$
$k_{0,1}$	min^{-1}	0.018 ± 0.011	0.00	$P < 0.06$
$k_{0,4}$	min^{-1}	0.39 ± 0.10	0.40 ± 0.02	n.s.
$k_{4,1}$	min^{-1}	0.10 ± 0.03	0.15 ± 0.01	n.s.
$k_{4,5}$	min^{-1}	0.128 ± 0.011	0.067 ± 0.019	$P < 0.05$
$k_{5,4}$	min^{-1}	0.34 ± 0.11	0.18 ± 0.02	n.s.

All parameters in the FFA part of the model ($k_{5,4}$, $k_{4,5}$, $k_{0,4}$, and FIB) were constrained to be the same for oleic acid injected as the albumin complex and derived from chylomicrons. The data for each rat were fitted separately and the values in the table are means $\pm$ SEM for the fitted parameters (n = 5). As some of the parameter groups were not normally distributed, Kruskal-Wallis test was used to test for significant differences.

plasma triglycerides 20 min after injection of chylomicrons was usually 4% or more of the injected dose. Hence, recirculation of label in endogenous lipoproteins could affect the last time point, 20 min, but usually not to any major extent. We have omitted the 20-min data point from the model calculations, but we have not included any pathway for recirculation of label in endogenous triglycerides. There was also a tendency for the first data point, (1 min) to fall below the predicted value. This will be dealt with below (see section on core label).

In this experimental setting, $k_{4,5}$, $k_{5,4}$, $k_{0,4}$, and FIB for FFA are defined mainly by the data for injected unesterified oleic acid. The model predicted that $k_{4,5}$, i.e., the rate for return of label from tissue lipids, was higher in fasted compared to fed rats (Table 1). Neither $k_{5,4}$, the rate of entry of label from FFA into tissue lipids, nor $k_{0,4}$, the rate for loss of label into storage pools or by oxidation, differed significantly between fasted and fed rats.

In fed rats it was necessary to allow some label to transfer out from chylomicron TG without mixing with the FFA, i.e., to add $k_{0,1}$ to the model (Fig. 5). The fraction that went out this way varied among the rats but as an average the fitted parameters predicted that it was about 20% (Table 1). In fasted rats $k_{0,1}$ adjusted to zero.

The rate for disappearance of TG label from blood ($k_{4,1}$ plus $k_{0,1}$) did not differ significantly between fed and fasted rats.

Comparison of the tissue distribution of label from FFA and from chylomicrons

The considerations above suggested that most of the label from chylomicron triglycerides mixed with the FFA pool. If mixing was complete, chylomicron-derived oleic acid should have the same metabolic fate as oleic acid from plasma FFA. To test this, labeled oleic acid was injected as the albumin complex or contained in chylomicron triglycerides to fed and fasted rats (Table 2). Twenty minutes later samples were taken from three sites: liver, heart, and epididymal fat. In fed rats, there was twice as much label in heart and almost five times more label in adipose tissue from chylomicrons. In fasted rats there was 1.5 times more label in the liver and four times more in the heart from chylomicrons. These clear differences show that there must be direct pathways to cellular metabolism, which do not involve complete mixing with plasma FFA. In the model this would be represented by the pathway out from compartment 1.

Model for core label

The next step in our analysis was to derive a model for the clearance of core label (Fig. 6). It is evident that there was a slow initial phase. Hence, the data are not consistent with monoexponential decay, as suggested in

TABLE 2. Tissue distribution of [^{14}C]oleic acid injected as the albumin complex compared to [^{3}H]oleic acid in chylomicrons

		[^{14}C]Oleic Acid as FFA		[^{3}H]Oleic Acid in Chylomicrons	
		Fed	Fasted	Fed	Fasted
		percent of injected dose			
Liver	per organ	22.2 ± 1.8	12.0 ± 0.5^b	22.2 ± 3.1	18.5 ± 0.6^d
Heart	per organ	0.40 ± 0.11	0.32 ± 0.03	0.83 ± 0.19	1.31 ± 0.18^d
Epididymal fat	per g tissue	0.50 ± 0.10	0.76 ± 0.06^a	2.38 ± 0.43^c	0.73 ± 0.06^b

The two labeled materials were mixed and injected to anesthetized rats. Twenty minutes later the rats were exsanguinated, tissue samples were taken, and the lipid radioactivity was determined as described in Materials and Methods. The data are means $\pm$ SEM for five rats in each group, and are calculated as indicated in the Table.
[a,b]Denotes a significant difference fed vs. fasted with $P < 0.06$ and $P < 0.01$, respectively (one-way ANOVA).
[c,d]Denotes a significant difference [^{14}C]oleic acid compared to [^{3}H]oleic acid with $P < 0.06$ and $P < 0.01$, respectively (paired t-test).
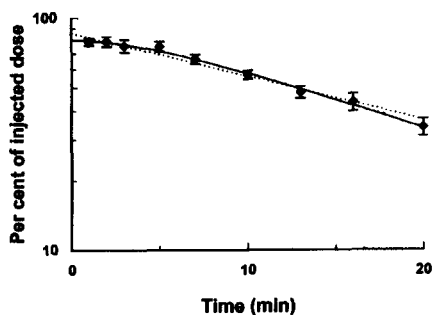
Fig. 6. Clearance of chylomicron core label. In this experiment the chylomicrons contained only core label. The clearing was slower than usual and the experiment therefore demonstrates the biphasic nature of the curve well. Observed values (◆, means ± SEM, n = 5). The dotted line shows the best fit to a monoexponential decay function. It is apparent that this does not adequately describe the first part of the curve. An open two-compartmental model, as shown in the lower panel, was needed for this. Compartments 11 and 12 represent label in chylomicrons/remnants in blood. The fit derived by this model is shown in solid lines. Model symbols as in Fig. 4.

some previous studies (7, 27, 28), but require a model where the core label must move into a second compartment before it can leave the blood rapidly. There was no feature in the data that required that label could leave the system directly from the first compartment. Such an open two-compartmental model fitted the data well (Fig. 6).

The rate for transfer from the first to the second compartment, $k_{12,11}$, is determined by the downward bend in the data. In our experiments samples were taken 1 and 2 min after injection of the chylomicrons. In most cases $k_{12,11}$ could be resolved, but sometimes there was too much noise in the data. In the experiment in Fig. 4, $k_{12,11}$ was 0.75 min^{-1}. In some experiments values as low as 0.3 min^{-1} were encountered whereas in other cases the rate adjusted towards infinity. The median value was ca. 0.7 min^{-1}. Thus, transfer of material from the first to the second compartment was rapid; the residence time for core label in the first compartment was almost always less than 2 min.

Chylomicrons containing both TG and core label

To analyze this type of data we joined the model for core label in Fig. 6 with the model for TG label and FFA in Fig. 5 (**Fig. 7**). The following assumptions were made. *I*) The disappearance of core label is not a selective process but TG label is transferred out by the same

pathway. Hence, analogous parameters should be applied to TG label ($k_{12,11}$ = $k_{2,1}$; $k_{13,12}$ = $k_{3,2}$). *II*) Both chylomicron compartments are hydrolyzed at the same rate ($k_{4,1}$ = $k_{4,2}$).

No labeled unesterified oleic acid was injected. Hence, this experiment does not give direct data for FFA turnover and these parameters could not be resolved. We therefore tried to use the mean values from Table 1. To accommodate differences between individual rats at least one parameter had to be unconstrained. For this, $k_{5,4}$ was allowed to adjust in the fitting process.

In this setting, $k_{12,11}$ and $k_{0,12}$ are determined almost entirely by the data on core label. Applying the same rate constants to TG label ($k_{2,1}$ and $k_{0,2}$) meant that about half of the triglycerides left blood with the core label
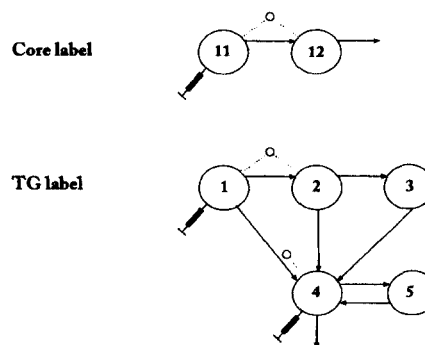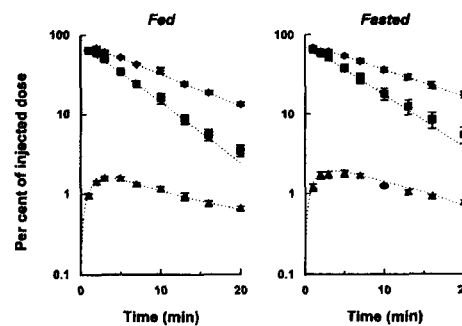

Fig. 7. Turnover of core and TG labeled chylomicrons. Doubly labeled chylomicrons were injected to anesthetized rats. The upper panels show the observed data for ^{3}H-core label (◆), ^{14}C-TG label (■), and ^{14}C-FFA (▲) in fed (left panel) and fasted (right panel) rats, respectively. The lines show the fit to the model in the lower panel. Compartments 11 and 12 represent core label in chylomicrons in blood. These are the same compartments as in Fig. 6. Compartments 1 and 2 are analogous compartments for TG label in blood, whereas compartment 3 represents TG label that has left the blood. Compartments 4 and 5 are the same as in Fig. 4. The data for individual rats (5 in each group) were fitted to the model. The mean values for the derived parameters are given in Table 3. The curves were generated from these mean parameters. The observed data are plotted as means ± SEM. Model symbols as in Fig. 4.

TABLE 3. Model parameters for the experiment in Fig. 7

		Fed	Fasted	Statistics
FIB (core and TG label)	fraction in blood	0.71 ± 0.03	0.72 ± 0.03	n.s.
FCR (core label)	min^{-1}	0.082 ± 0.003	0.070 ± 0.004	$P < 0.06$
FCR (TG label)	min^{-1}	0.158 ± 0.011	0.140 ± 0.017	n.s.
Part of TG label via $k_{3,2}$	fraction	0.48 ± 0.04	0.49 ± 0.04	n.s.
$k_{2,1}$	min^{-1}	0.75 ± 0.10	0.66 ± 0.11	n.s.
$k_{3,2}$	min^{-1}	0.093 ± 0.002	0.077 ± 0.005	$P < 0.05$
$k_{4,1}$	min^{-1}	0.083 ± 0.011	0.075 ± 0.015	n.s.
$k_{4,3}$	min^{-1}	0.63 ± 0.19	3.3 ± 2.1	n.s.
$k_{5,4}$	min^{-1}	0.93 ± 0.17	0.85 ± 0.21	n.s.

All parameters except $k_{5,4}$ in the FFA part of the model ($k_{4,5}$, $k_{0,4}$, and FIB) were set to the values from Table 1. Transfer from the first to the second chylomicron compartment ($k_{12,11}$ and $k_{2,1}$) and for transfer out of blood from the second compartment ($k_{0,12}$ and $k_{3,2}$) were constrained to be the same for core and TG label. The rates for direct transfer of material from TG label in the first and second chylomicron compartments to FFA ($k_{4,1}$ and $k_{4,2}$) were constrained to be equal. The data for each rat were fitted separately and the values in the Table are means $\pm$ SEM for the fitted parameters (n = 5). As some of the parameter groups were not normally distributed, Kruskal-Wallis test was used to test for significant differences.

($k_{0,2}$) and only about half went to the FFA pool ($k_{4,1}$ and $k_{4,2}$). This gave values for label in FFA that were too low. To remedy this we introduced a compartment 3 for the TG label that left blood and a pathway from this compartment to the FFA ($k_{4,3}$). This can be envisioned as hydrolysis of triglycerides that have left blood with the core label. In fasted rats the fitting process wanted to transfer all TG label from compartment 3 into the FFA pool by imparting a high $k_{4,3}$. In fed rats this would give too much label in plasma FFA. There were two possibilities to remedy this. One was to allow $k_{4,3}$ to have a lower value in fed than in fasted rats, so that TG label built up in compartment 3. The other possibility was to set $k_{4,3}$ to the same value as in fasted rats but add a pathway out from compartment 3 ($k_{0,3}$). The data did not contain information that allowed us to distinguish between these two alternatives. In the first alternative, the parameters predicted that at the end of the experiment, 20 min, about 16% of the TG label remained in compartment 3. To test the other alternative we assigned values from 0.5 to 16 min^{-1} to $k_{4,3}$, and let the program calculate $k_{0,3}$. Throughout that range, the ratio $k_{0,3}$ to $k_{4,3}$ was nearly constant at 1:3. Hence, both alternatives predicted that in fed rats about one-fifth of the TG label that left blood with the core label did not reappear in the FFA. This is the same conclusion as reached from the analysis in Fig. 5.

The rate constants for transfer of core label out from blood ($k_{0,12}$) and for direct transfer of TG label to the FFA pool ("lipolysis", $k_{4,1}$ and $k_{4,2}$) did not differ significantly between the nutritional states (**Table 3**). These parameters could be constrained to be the same for fed and fasted rats without markedly impairing the fit. Thus, the difference between the nutritional states was in the turnover of FFA, and in the extent to which chylomicron TG label reappeared in the FFA pool.

From the derived parameters (Table 3) it can be calculated that the time at which the ratio between TG and core label in blood had dropped to 0.5, i.e., when the chylomicrons had lost half of their triglycerides through lipolysis, was 9.1 ± 1.1 min in fed rats and 10.1 ± 1.9 min in fasted rats. At this time $64 \pm 4\%$ and $62 \pm 3\%$, respectively, of the core label had been removed from blood. Thus, a large fraction of the core label was removed from blood while the average chylomicron still retained more than half of its triglycerides.

Tissue distribution of core and TG label from injected chylomicrons

The model in Fig. 7 assumes that TG label follows core label out from plasma. We therefore studied how the TG and core labels distributed between the tissues (**Table 4**). In this experiment more than 95% of the labels had disappeared from blood 20 min after injection of the chylomicrons. All tissues were analyzed. Total recovery was 76% for the core label and 59% for the TG label.

There was significant core label in all tissues over what could be contributed by remaining blood. The three tissues that contained most of the label were liver, muscles, and white adipose tissue (including the skin). Forty-four percent of the core label but only 10% of the TG label was in the liver. This indicates that the liver, as expected, had taken up lipolyzed chylomicrons. In muscles and in adipose tissue the relation between the labels was reversed; there was more TG than core label, as expected from the action of lipoprotein lipase. Brown fat displayed the most selective uptake of fatty acid radioactivity, which was ten times higher than the core label.

Hussain et al. (16) reported a high uptake of core label from chylomicrons/chylomicron remnants in the bone marrow and interpreted this in terms of endocytosis of chylomicrons by cells of the reticulo-endothelial system. In our study, only 0.6% of the core label was found in the combined skeleton of the upper and lower extremities, sites rich in bone marrow. There was 3.1% of the

TABLE 4. Tissue distribution of label from chylomicrons in fed rats

		Core Label	TG Label
		percent of injected dose	
Blood	per organ	3.1 ± 0.9	1.6 ± 0.8
Liver	per organ	43.5 ± 7.2	9.8 ± 3.1
Brown fat	per organ piece	0.05 ± 0.01	0.50 ± 0.08
Fat	sum of all pieces	2.6 ± 0.2	7.2 ± 0.8
Skin	sum of all pieces	3.6 ± 0.4	6.0 ± 0.3
Heart	per organ	1.2 ± 0.3	1.0 ± 0.3
Muscles	sum of all pieces	12.1 ± 0.9	19.8 ± 4.0
Lung	per organ	0.78 ± 0.18	0.51 ± 0.11
Spleen	per organ	3.1 ± 0.4	1.6 ± 0.4
Gastrointestinal organs	sum of all pieces	1.5 ± 0.8	2.6 ± 0.4
Head	sum of all pieces	2.5 ± 0.5	6.8 ± 0.5
Anterior extremity skeleton	sum of all pieces	0.23 ± 0.02	0.22 ± 0.02
Posterior extremity skeleton	sum of all pieces	0.33 ± 0.04	0.39 ± 0.03
Other tissues	sum of all pieces	2.1 ± 0.3	2.4 ± 0.4
Total recovery		76 ± 9	59 ± 6

Doubly labeled chylomicrons were injected to anesthetized fed rats. Twenty min later the rats were exsanguinated and cut into pieces according to anatomical borders. This gave a total of 140 pieces weighing 0.04–25 g. The pieces were saponified and lipid-soluble material was extracted as described in Materials and Methods. The data are means ± SEM for five rats in each group, and are calculated as indicated in the table.

core label in the spleen, but only 0.8% in the lungs. Overall, the bone marrow and the reticulo-endothelial system do not appear to be major sites for uptake of chylomicron core lipids in the rats we studied.

It is often assumed that nearly all core lipids end up in the liver with the chylomicron remnants. In contrast, our data show that after 20 min about 30% of the core label had located in other tissues, while the liver and blood contained about 47% (Table 4).

We then studied the effects of nutrition on the distribution of label into some selected tissues (**Table 5**). There were marked effects only in adipose tissue and heart. In both cases the differences went in the same direction for core and TG label, more in adipose tissue and less in heart in the fed compared to the fasted rats. In the other tissues the differences were small or statistically not significant.

Reanalysis of emulsion clearance

In a previous article, some of us studied the metabolism of an intravenous lipid emulsion compared to chylomicrons (3). Both the emulsion and the chylomicrons contained a TG label and a core label but no labeled unesterified fatty acid was injected. We first used the same approach as in Fig. 7, i.e., to use the values from Table 1 for the FIB for FFA and $k_{4,5}$ and $k_{0,4}$, but let $k_{5,4}$ free to adjust for differences between individual rats. We found, however, that no major adjustments of $k_{5,4}$ were needed for these datasets and we therefore set all parameters in the FFA part of the model ($k_{4,5}$, $k_{5,4}$, $k_{0,4}$, and FIB for FFA) to the mean values in Table 1. The remaining parameters could be fitted for each individual rat so that the model described the data well.

In rats given emulsion the clearance from plasma was monoexponential for both core and TG label. Therefore

TABLE 5. Effect of nutritional state on the tissue distribution of core and TG label

		TG Label		Core label		TG Label / Core label	
		Fed	Fasted	Fed	Fasted	Fed	Fasted
		percent of injected dose					
Liver	per organ	21.6 ± 0.9	16.2 ± 0.9[b]	57.7 ± 1.0	51.5 ± 2.2[a]	0.38 ± 0.02	0.32 ± 0.02
Spleen	per organ	0.94 ± 0.05	1.03 ± 0.09	1.81 ± 0.12	1.96 ± 0.13	0.52 ± 0.02	0.53 ± 0.02
Lung	per organ	0.60 ± 0.07	0.51 ± 0.04	0.52 ± 0.02	0.63 ± 0.04[a]	1.14 ± 0.10	0.81 ± 0.03[a]
Heart	per organ	0.84 ± 0.15	1.40 ± 0.14[a]	0.43 ± 0.03	1.00 ± 0.11[c]	1.90 ± 0.29	1.42 ± 0.08
M soleus	per g	0.87 ± 0.06	1.08 ± 0.14	0.44 ± 0.04	0.61 ± 0.07[a]	2.00 ± 0.17	1.80 ± 0.18
M gastrocnemius	per g	0.20 ± 0.01	0.25 ± 0.01[a]	0.11 ± 0.01	0.12 ± 0.01	1.88 ± 0.13	2.21 ± 0.14
Kidney	per organ	0.30 ± 0.01	0.32 ± 0.01[a]	0.19 ± 0.01	0.20 ± 0.01	1.61 ± 0.07	1.62 ± 0.11
Epididymal fat pad	per g	1.32 ± 0.21	0.42 ± 0.08[b]	0.27 ± 0.04	0.16 ± 0.01[a]	4.95 ± 0.55	2.72 ± 0.24[b]

Doubly labeled chylomicrons were injected to anesthetized rats that either had free access to food or were fasted overnight. Twenty minutes later the rats were exsanguinated, tissue samples were taken, and the lipid radioactivity was determined as described in Materials and Methods. The data are means ± SEM for five rats in each group, and are calculated as indicated in the table.
[a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$ for fed compared to fasted rats.

$k_{12,11}$ always adjusted to the upper limit. Hence, there was no need for more than one compartment for core or TG label in blood. In contrast there was a distinct lag phase in rats given chylomicrons; $k_{12,11}$ was 0.86 ± 0.16 min^{-1} in fed rats and 1.88 ± 1.27 min^{-1} in fasted rats (n.s.). Direct lipolysis ($k_{4,1}$ and $k_{4,2}$) was much slower in rats given emulsion, 0.016 ± 0.004 min^{-1} and 0.025 ± 0.004 min^{-1} compared to 0.079 ± 0.021 min^{-1} and 0.057 ± 0.007 min^{-1} for rats given chylomicrons, fed and fasted, respectively ($P < 0.001$ for emulsion vs. chylomicrons). Transfer of TG label that had left blood with the core label into the FFA pool was also slower in the rats given emulsion; $k_{4,3}$ was 0.159 ± 0.031 min^{-1} and 0.078 ± 0.013 min^{-1} compared to 0.28 ± 0.12 min^{-1} and 0.99 ± 0.01 min^{-1} in rats given chylomicrons, fed and fasted, respectively ($P < 0.001$ for emulsion vs. chylomicrons). It was not possible to set $k_{4,3}$ to zero in the rats given emulsion. This would have given too low levels of label in FFA. The fraction of TG label that left blood together with the core label was much higher in rats given emulsion than in rats given chylomicrons, 0.86 ± 0.02 and 0.79 ± 0.03 compared to 0.52 ± 0.05 and 0.57 ± 0.04, fed and fasted, respectively ($P < 0.001$ for emulsion vs. chylomicrons). The amount of TG label that appeared in the FFA pool via $k_{4,1}$ and $k_{4,2}$ within 20 min was $47 \pm 6\%$ and $50 \pm 12\%$ in rats given chylomicrons but only $13 \pm 2\%$ and $20 \pm 3\%$ in rats given emulsion, fed and fasted, respectively.

DISCUSSION

In this paper we have used compartmental models to better understand the pathways of chylomicron clearance. Such models have been extensively used to analyze lipoprotein kinetics in humans (5, 6), and have usually focused on the pathways taken by the particles and the apolipoproteins. Here we have turned our attention towards the lipids and have based our analyses on the kinetics of disappearance from blood and the tissue distribution of the labeled lipids.

Both core and TG label distributed into volumes that were significantly larger than the blood volume. As the two labels behaved similarly in this respect it is likely that it was the entire chylomicron that distributed into the larger apparent volume. Some of this may be tissue spaces into which the chylomicrons have immediate access, such as the spaces of Disse in the liver (29). Some must represent `margination' of chylomicrons to endothelial binding-lipolysis sites (30). Harris and Harris (28) found that with increased amounts of injected chylomicrons, the distribution volume decreased. This suggests that the number of margination sites is limited. In a recent study by Karpe and Hultin (4), the clearance of chylomicrons from blood was competed out by clamping the TG level at 8 mmol/l by infusion of a fat emulsion. We have analyzed their data using the model developed here. The FIB for TG and core label increased from 0.80 ± 0.03 in the controls to 0.88 ± 0.01 in triglyceride-clamped rats ($P < 0.05$), showing that some of the margination was competed out.

In the modelling it was necessary to include at least two compartments for chylomicrons in blood. The feature of the data that necessitated this was the earliest time points, which showed that the rate of disappearance for core label was initially very slow, except for the distribution effect. The injected chylomicrons must represent a mixture of particles differing in size and perhaps other properties. This heterogeneity cannot, however, explain the initial delay in clearing. Nonhomogeneity could give rise to a rapid first exponent and a slower second exponent but not to a delay. Hence, chylomicrons, as injected, must transfer into a second model compartment before the core label can leave the blood at a significant rate. This is in concert with the concept that chylomicrons must be transformed into remnants before they are cleared (31, 32). The transit time was, however, short. For some of the individual rats we could not define the rate from the limited data, only set a lower limit. It is clear, however, that the average chylomicron spent less than 2 min in compartment 1 before it transferred into the second model compartment, i.e., the rate of `remnant' formation was high.

Lipolysis was modelled to occur from the blood compartments for chylomicron TG label. There is, however, very little lipoprotein lipase in the circulating blood (33) and its contribution to lipolysis must be small. It follows that most of the lipolysis must be pictured as occurring on chylomicrons that have temporarily left the blood for endothelial lipolysis sites, but will return, i.e., are in reversible 'equilibrium' with the chylomicrons circulating in blood. At the particle level, lipolysis is unlikely to be a first order process, more likely it is a zero order process. The kinetics of the removal was, however, exponential. Hence, it is probably not lipolysis as such that is rate-limiting, but rather the interaction of the particles with the endothelial binding-lipolysis sites. In support of this, lipolysis is greatly accelerated when the lipase is released into the circulation by heparin (1).

In the model for FFA turnover, derived from injection of albumin-bound oleic acid, the label distributed into a volume three to four times larger than the blood volume. It would have been possible to set the distribution volume of the initial FFA compartment equal to the blood volume, and add another compartment that equilibrated almost instantaneously with the FFA in blood. This would not have served any useful purpose

and we chose to let the FFA compartment have a distribution volume larger than the blood volume. The extravascular part of the `FFA pool' presumably represents fatty acids on their way to, or from, metabolic sites in cells.

A large fraction of the oleic acid from chylomicron triglycerides reappeared in the FFA pool. This conclusion does not depend on the design of the model but is given by the area under the curve for label in plasma FFA and the assumption that label that enters this pool from chylomicron lipolysis behaved in the same manner as label injected as albumin-bound fatty acid. In fasted rats the fraction actually approached, and could not be distinguished from, 100%. The fact that the distribution volume for the FFA pool was larger than the blood volume means that only one-quarter to one-third of the fatty acids were actually in blood. Our interpretation is that lipolysis at endothelial, and perhaps other, sites generated fatty acids into the parts of the FFA pool not in plasma, and that the fatty acids then equilibrated from there with plasma. Hence, what the model says is that virtually all of the chylomicron-derived fatty acids mixed into the same pools as label administered as albumin-bound FFA did; the model does not say that all of the chylomicron-derived fatty acids actually moved between tissues via plasma. Several earlier studies show that a large fraction of chylomicron-fatty acids are released into plasma. Scow (34) perfused adipose tissue of fed rats. About 55% of the fatty acids from the chylomicron triglycerides metabolized by the tissue were incorporated into tissue lipids while the rest, 45%, was released as FFA to the medium. In other experiments the perfused mammary tissue of lactating rats (34). The tissue metabolized about 12% of the perfused triglycerides. About 65% of this was retained in tissue lipids, while about 35% was released as FFA to the medium. During lipid absorption in humans the fatty acid composition of plasma FFA changes in the direction of the dietary lipids, demonstrating that a large proportion of the FFA are derived from lipolysis of chylomicron triglycerides (33). From studies on the metabolism of VLDL and chylomicrons over adipose tissue in humans, Frayn and colleagues (35) concluded that in some situations the adipose tissue breaks down circulating lipoprotein triglycerides to circulating FFA and glycerol without storing much of the material. They suggested that these fuels are metabolized elsewhere in the body, perhaps at sites less able to clear chylomicrons.

The implication would be that lipolysis is needed to release fatty acids from the triglycerides, but after that the metabolism of chylomicron-derived fatty acids does not differ from that for plasma FFA. This forced us to reinvestigate what differences in tissue distribution there are between label administered in the two forms.

Preliminary experiments indicated that the differences were small in most tissues, but it was also clear that in some tissues there were clear differences as first demonstrated by Bragdon and Gordon (36). Two examples were the heart and the adipose tissue. In the heart a larger fraction of label was found after chylomicrons than after FFA in both nutritional states. In the adipose tissue there was nearly five times more label from chylomicrons than from FFA in the fed state. These are classical examples of the directive effect of lipoprotein lipase activity. In quantitative terms the really significant difference would be adipose tissue in the fed state, where our data suggest that 20–30% of the chylomicron-derived fatty acids locate under the present conditions. Detailed comparisons are not possible as we have no measures of oxidation in the tissue, only data for what was retained in lipid esters. Cryer et al. (37) found a close correlation between the lipoprotein lipase activity in rat epididymal adipose tissue and the uptake of fatty acids from labeled chylomicrons. There is net extraction of triglycerides by adipose tissue in humans but not of FFA (38). Hence, it is clear that lipoprotein lipase, and perhaps other mechanisms, does drive some of the fatty acids from chylomicrons into tissues in a proportion that differs from the uptake of FFA. The present data suggest that this occurs only for a limited proportion of the fatty acids, a large fraction mixes into and shares the metabolic fate of plasma FFA.

A limitation is that we used only labeled oleic acid. Different fatty acids have different transport rates in the FFA fraction and different metabolic fates in tissues (39, 40). The rationale for using oleic acid was that it is a common fatty acid that is incorporated almost exclusively into the triglycerides of chylomicrons. One should be cautious about extrapolating the data to other fatty acids; there may be more pronounced differences in how, for instance, long polyunsaturated fatty acids are handled from chylomicrons compared to from plasma FFA, than found here for oleic acid.

Lipoprotein lipase hydrolyzes chylomicron triglycerides to fatty acids and 2-monoglycerides. These are presumably split by the monoglyceride hydrolase that is present in many cell types (41). The monoglycerides were not accounted for by separate compartments in our model. In perfusion experiments Scow (34) noted that free glycerol began to appear in the medium somewhat later than FFA appeared. He interpreted this as evidence that hydrolysis of the monoglycerides occurred after they had moved into tissue cells, whereas formation of monoglycerides took place at the endothelium, in vesicles traversing the endothelium and in the subendothelial space.

Application of the model to the clearance of a doubly labeled lipid emulsion showed that there were major

differences between the way the emulsion and chylomicrons are handled in vivo. The clearance of emulsion core label from the blood started immediately and there was no need for a two-compartmental model in plasma. This indicates that the emulsion droplets were immediately available for removal from the circulation. This was strikingly different from chylomicron metabolism where a two-compartmental model was necessary.

A central question is how the core label left the circulation. One pathway indicates that retinyl esters and other core lipids remain with the chylomicron as most of the triglycerides are removed by lipoprotein lipase-mediated hydrolysis, and that the core lipids only leave the circulation with extensively lipolyzed remnants (32). This process is assumed to occur by receptor-mediated endocytosis, largely in the liver. This certainly occurred in our experiments and presumably accounted for most of the retinyl label that was found in the liver. Here, the ratio of fatty acid to retinol radioactivity at 20 min was below 0.4, in concert with uptake of lipolyzed remnants. It is unlikely, however, that remnant uptake accounted for all of the retinyl ester removal. I) The core label started to disappear virtually immediately after injection; there was a lag phase but it was very short. If extensive lipolysis was needed before the chylomicrons were recognized for uptake, the lag phase should have been much more apparent. From the model we could calculate that more than 60% of the core label left blood while the chylomicrons retained more than half of their triglycerides. II) Retinol radioactivity was found in all tissues, whereas remnant removal is supposed to occur almost exclusively in the liver. In the experiment in Table 1, about 3% of the retinol label remained in blood after 20 min, 44% was in the liver, 29% in other tissues, and 24% had been lost, presumably through oxidation. This is not consistent with the view that virtually all chylomicron core components end up in the liver with remnants. We have recently summarized data from the literature and found that in most of the reported studies a rather large proportion of core components from injected chylomicrons were taken up elsewhere than in the liver (3). III) In all tissues except liver and spleen, the ratio of fatty acid to retinol radioactivity was above 1.

The other possibility is that retinyl esters are transferred out from the lipoproteins into tissues. There is earlier evidence for such a process. Fielding (42) studied the metabolism of chylomicrons in perfused rat hearts and found that a relatively large fraction of cholesteryl esters were taken up by the heart. In contrast, no apolipoprotein B-48 was removed from the medium. For instance, in one set of experiments apolipoprotein B-48 radioactivity in the medium after 15 min perfusion was 98% of initial under conditions where mean triglyceride removal was 60% and mean cholesteryl ester

removal was 33%. He found that the uptake of cholesteryl esters was virtually abolished by heparin. Some of the already bound cholesteryl ester radioactivity could be released back to the medium by heparin, but after a few minutes of chase perfusion the radioactivity became heparin-insensitive. These data suggest initial binding of the chylomicrons to heparin-sensitive sites, perhaps involving lipoprotein lipase, soon followed by transfer of core lipids into the tissue. Scow (34) perfused chylomicrons through mammary and adipose tissues of rats. In both cases, other lipids were extracted together with triglycerides by the tissue, and the process appeared to depend on the amount of lipoprotein lipase present. Chajek-Shaul et al. (43) and Friedman et al. (44) have studied the mechanism for transfer of core lipids from chylomicrons to cultured cells using non-hydrolyzable ether analogues of cholesteryl esters. Addition of exogenous lipoprotein lipase enhanced the uptake many-fold in all cell types. For this to occur, the enzyme had to be bound to the cell surface, presumably via heparan sulphate, but did not have to hydrolyze any lipids. A closer look at our data shows a clear effect of the nutritional state on the distribution of the retinyl label into heart and adipose tissue. These tissues are classical sites for nutritional regulation of functional lipoprotein lipase activity. In both cases there was more retinol label in the up-regulated state, i.e., more in adipose tissue of fed compared to fasted rats and more in heart of fasted rats. This suggests a relation between lipoprotein lipase activity, fatty acid uptake, and uptake of retinol label, as in the data of Fielding, Scow, Chajek-Shaul et al., and Friedman et al. (34, 42–44). The detailed mechanism is not clear, but may relate to the process by which cholesteryl esters are transferred into cells from HDL without the lipoprotein particles itself entering the cells (45–48).

Blaner et al. (12) recently reported that lipoprotein lipase can hydrolyze retinyl esters, questioning their use as core label. We reinvestigated this, using chylomicrons as substrate. With moderate amounts of lipoprotein lipase added there was no hydrolysis of retinyl esters. It was only at very high amounts of added lipase, similar to those used by Blaner et al., that retinyl ester hydrolysis was seen. Even so, only one-third of the retinyl esters were hydrolyzed when over 90% of the triglycerides were removed. If lipoprotein lipase-mediated hydrolysis was a major event in the removal of retinyl esters this would have to occur during the first 10 min when about 60% of the retinyl esters were removed. At this time the average chylomicron had undergone rather limited lipolysis and retained more than half of its triglycerides. We do not believe that hydrolysis by lipoprotein lipase accounts for the removal of retinyl esters from the blood.

The approach taken here has been to search for

quantitative estimates of how lipids are channelled from chylomicrons into tissues. Some of the answers run counter to prevailing opinion; the large fraction of fatty acids that mix into the FFA pool, the quick removal of core lipids, and the large fraction of core lipids found in tissues outside the liver. The analyses should not be taken as final firm figures, but should be seen as a way to probe the implications of currently favored hypotheses, point at inconsistencies, and indicate areas for further experiments and analyses.■

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