Metabolism of emulsions containing medium- and long-chain triglycerides or interesterified triglycerides

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Abstract This study compares the clearing and metabolism of three different lipid emulsions. They had the same phospholipid emulsifier and similar particle sizes. In one (LLL) the core component was long-chain triglycerides (TG), the second (MMM/LLL) contained equal molar amounts of medium- and long-chain TG, the third (MLM) contained synthetic TG with medium-chain (M) fatty acids in the 1,3-positions and a long-chain (L) fatty acid in the 2-position. In model experiments with bovine lipoprotein lipase, the MMM component was hydrolyzed preferentially in the MMM/LLL emulsion so that the initial products were M fatty acids and M monoglycerides. The MLM emulsion, in contrast, gave M fatty acids and formation of L-MG (monoglyceride) throughout hydrolysis. For in vivo studies [³H]oleic acid was incorporated into the emulsion TG as marker for the long-chain component. 🔲 After bolus injection to rats, the MMM/LLL and MLM emulsions were cleared more rapidly than the LLL emulsion. This was true at all TG loads studied (4–64 mg for a 200 g rat). The labeled oleic acid was oxidized somewhat more rapidly when administered in the MLM emulsion compared to the MMM/LLL emulsion. There were only slight differences in tissue distribution of label. Hence, differences in in vivo metabolism of the long-chain fatty acids were small compared to the marked differences in TG structure and in patterns of product release during in vitro lipolysis.— **Hultin, M., A. Müllertz, M. A. Zundel, G. Olivecrona, T. T. Hansen, R. J. Deckelbaum, Y. A. Carpentier, and T. Olivecrona.** Metabolism of emulsions containing medium- and long-chain triglycerides or interesterified triglycerides. *J. Lipid Res.* 1994. **35:** 1850–1860.

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Lipid emulsions are widely used to provide energy substrate and essential fatty acids for patients (1–3). An important question for rational design of such emulsions is what the fatty acid composition of the triglycerides (TG) should be. Presently the most used emulsions are based on TG from soy oil which contain more than two-thirds polyunsaturated fatty acids, mainly linoleic acid. Concerns have been raised about this, and it has been suggested that the content of polyunsaturates should be reduced (4–6). One way is to use mixtures of long-chain and medium-chain fatty acids. For this there are two possibilities, to mix medium-chain and long-chain triglycerides or to use triglycerides containing a mixture of fatty acids in the same molecule.

It is generally assumed that fat emulsions are metabolized by lipoprotein lipase (LPL) by mechanisms similar to those for endogenous chylomicrons (1, 7). Experiments in vitro have shown that in emulsion particles containing medium-chain and long-chain TG, the medium-chain component is hydrolyzed more rapidly, and the remnant particles become enriched in long-chain TG (8). It would therefore be expected that in vivo a relatively large proportion of long-chain TG administered in such emulsions would be delivered to the liver in the remnant particles (7, 9). Another aspect is that LPL shows positional specificity for the 1,3 positions (10–12). Hence, the primary products of lipolysis are fatty acids and 2-monoglycerides. Available data indicate that monoglycerides recirculate in plasma to a lesser degree than fatty acids (13). This suggests that the 2-monoglyceride might partition more towards the tissue where lipolysis takes place, and hence the metabolic fate of that fatty acid might differ from the fate of the 1,3 fatty acids.

It has been suggested that long-chain fatty acids exert feed-back regulation of LPL action in vivo (14–16). The molecular basis for this is that the enzyme binds the fatty acids. Medium-chain fatty acids do not have these effects. Hence, the metabolism of emulsions containing medium-chain fatty acids might be exempt from some of the constraints imposed on chylomicron metabolism by fatty acid control of LPL. This has not been directly tested, in vitro or in vivo.

The purpose of this study was to explore how the type of fatty acid, medium- or long-chain, and the TG structure influences the metabolism of fatty acids contained in TG-rich particles. For this we used three emulsions. One represented the common type of emulsions with long-chain TG as the core component. The second emulsion contained equal moles of medium- and long-chain TG, whereas the third emulsion contained synthetic TG with medium-chain fatty acids in the 1,3-position and a long-chain fatty acid in the 2-position. Hence, the overall compositions of these two emulsions were similar, but the TG structure was different. We first studied the patterns of product release from the different emulsions during in vitro lipolysis by LPL, and then how the emulsions were cleared after bolus injection to rats, the rate of oleic acid oxidation from the different emulsions, and the deposition of labeled oleate in tissue lipids.

MATERIALS AND METHODS

Materials

LPL was purified from bovine milk by chromatography on heparin-agarose (17). Apolipoprotein C-II (apoC-II) was isolated from human plasma (17). Bovine serum albumin (fraction V powder) was from Sigma, St. Louis, MO. Heparin was from Lövens, Malmö, Sweden.

TG-rich particles

Lipid emulsions were produced by the Microfluidizer® technique. They contained 20% TG, 1.2% phospholipids

TABLE 1. Fatty acid composition of emulsion triglycerides

Fatty Acid	LLL	MMM/LLL	MLM
		mol %	
8:0		32.8	31.5
10:0		19.8	18.4
16:0	10.9	1.8	5.5
18:0	3.6	0.6	1.7
18:1	24.0	11.4	12.0
18:2	50.0	29.1	25.1
18:3	7.8	3.4	3.9

Data are for the emulsions used for studies on product patterns during in vitro lipolysis. The fatty acid patterns were analyzed by capillary gas chromatography as described in Materials and Methods.

TABLE 2. Fatty acid composition of the 2-position in the emulsion TG

Fatty Acid	LLL	MMM/LLL	MLM
		mol %	
8:0		25.2	2.6
10:0		17.3	2.3
16:0	0.8	0.4	0.9
18:0	0.8	0.2	0.3
18:1 (n–9)	23.4	12.9	22.1
18:2 (n–6)	67.2	39.7	64.3
18:3 (n–3)	7.8	4.4	7.6

Data are for the same emulsions as in Table 1. The analyses were done as described in detail in Materials and Methods by enzymatic digestion, separation of products by thin-layer chromatography, followed by gas chromatographic analysis of the fatty acids in each fraction.

(Lipoid AG, Ludwigshofen, Germany), and 2.5% glycerol. The TG were either from soy bean oil (LLL), medium chain (MMM, DS Industries, Copenhagen, Denmark), or synthetic triglyceride (MLM). MLM was synthesized from soy bean oil and ethyl esters of medium-chain fatty acids by use of an immobilized 1,3-specific lipase (Lipozyme, NOVO Nordisk A/S) (18). As a consequence of the strict positional specificity of the lipase used, these TG contained predominantly medium-chain fatty acids in the 1,3 positions and long-chain fatty acids in the 2-position. One emulsion contained only LLL; one emulsion contained a mixture of equal molar amounts of LLL and MMM. The third emulsion contained only MLM. The fatty acid compositions of the emulsion TG are shown in **Table 1.** Mean molecular weights were 871 for LLL, 687 for MMM/LLL, and 731 for MLM. The positional distribution of the fatty acids was analyzed by enzymatic methods. For MLM the 2 position contained 95.1% long chain fatty acids and 4.9% medium-chain fatty acids (**Table 2**). Positions 1 and 3 contained 81.5% medium-chain fatty acids and 18.5% long-chain fatty acids.

Three sets of emulsions were prepared for this study. One set was used for preliminary studies on rates of in vitro lipolysis (data not shown). Another set was used for studies of product patterns during in vitro lipolysis (see Table 3 and Figs. 1 and 2). A third set was radiolabeled and used for studies on in vivo metabolism in rats. This latter set of emulsions contained 10% TG, 1.2% phospholipids, and 2.5% glycerol. For this, a trace amount of [³H]oleic acid-labeled triolein (prepared by Lennart Krabich, Department of Physiological Chemistry, Lund, Sweden) was incorporated in the LLL and MMM/LLL emulsions. Labeled M-[³H]L-M TG was made by chemical synthesis. The labeled MLM was mixed with enzymatically prepared bulk MLM TG and used to prepare a MLM emulsion for the in vivo experiments.

Synthesis of labeled M-[³H]L-M TG

The synthesis of 1,3-di(octanoyl/decanoyl)-2-[9,10-³H]oleoyl-glycerol was carried out in three steps according to published procedures (19–23). The reaction of octanoyl and/or decanoyl chloride on glycerol (1st step) gave a mixture of monoglycerides, 1,2-diglycerides, 1,3-diglycerides, and TG. The 1,3-diglycerides were separated by chromatography on silica gel impregnated with 5% boric acid (24–26). After two chromatographies the product was more than 98% pure 1,3-diglycerides with less than 1% 1,2-diglycerides (as determined by capillary gas chromatography on a Hewlett-Packard 5890 A equipped with a 60 × 0.25 mm Chromopack SPB-1 column). The final yield was 20% and the molar ratio of octanoyl to decanoyl acyl chains in the 1 and 3 positions was 53 to 47.

The diglycerides thus obtained were reacted with [³H]oleoyl chloride, prepared from [³H]oleic acid and oxalyl chloride (21–23), yielding 1,3-di(octanoyl/decanoyl)-2-[9,10-³H]oleoyl-glycerol after chromatography on 5% boric acid-impregnated silica gel as described above. The recovery was 85% in the last step. According to ¹³C-NMR (27) no unsaturation was present in the 1,3-positions of the TG. This means that no isomerization occurred during the reaction. In the 2-position an unsaturated/saturated ratio of 4/1 was found. This is in good agreement with the composition of the fatty acids used for the long-chain position. The final TG had a specific activity of 10.8 mCi/g.

In vitro lipolysis

Preliminary experiments were carried out to explore the properties of the experimental emulsions. Hydrolysis of all three emulsions was stimulated by apoC-II, with no marked differences in the extent of stimulation or in the amount of C-II needed. Maximal (initial) rates of lipolysis were attained at TG concentrations ≥ 2 mg/ml in buffer–albumin systems, and ≥ 4 mg/ml when the reactions were carried out in whole rat plasma. Compared to the rates observed with the LLL emulsion, the rates were 2.0- to 2.6-fold higher with the MMM/LLL and 1.6- to 1.9-fold higher with the MLM emulsions.

From these results we decided to use the following conditions for the studies on product patterns. The reactions were carried out in a pH-stat at pH 7.4 and 25°C using 0.05 M NaOH for titration. Enough LPL was added to attain approximately 5% hydrolysis of available ester bonds in 8–12 min. The medium was 5 ml 0.1 M NaCl containing per ml: 10 mg TG (0.025 ml 20% lipid emulsion), 1.25 mg glycerol, and 10 μg heparin. No apoC-II was added.

For experiments with the LLL emulsion, 50 mg fatty acid-free albumin was added per ml to provide a fatty acid acceptor. About 0.15 ml 0.05 M NaOH was needed to adjust the initial pH to 7.4. After the reaction the lipids were extracted according to Dole (28) and the lipid extract was taken to dryness.

For experiments with the MLM emulsion no albumin was added. This was based on preliminary experiments which showed that albumin had little effect at the levels of lipolysis studied (about 5%), but complicated the extraction procedure. After the reaction, 20 ml absolute ethanol, 1 ml acetic acid, and 1 ml internal standard (C 13:0 fatty acid) were added. The solvent was evaporated, a further 20 ml ethanol was added, and evaporation was continued until dryness. This extraction procedure was verified by a mass balance between the original TG and the different fractions present after hydrolysis.

The extracted lipids were redissolved in 3 ml trichloroethane and filtered through a 0.5 μm filter. An aliquot (0.7 ml) of the lipid extract was applied as a narrow band on a thin layer plate. The solvent used was n-heptane–diethylether–acetic acid 60:40:2. The lipid fractions were visualized by 2,7-dichlorofluorescein (0.2% in absolute ethanol). Bands corresponding to TG, fatty acids, diacylglycerols, and monoglycerides were scraped off. Tri-, di-, and monoglycerides were trans methylated by 0.5 N sodium methoxide in methanol, while the fatty acids were methylated by 1% sulfuric acid in methanol. The methylated samples were analyzed by gas chromatography on a HP5890 GC using a Supelco™ 10 fused silica capillary column (30 m: 0.25 mm ID).

Procedures for in vivo experiments

Male outbred Sprague-Dawley rats weighing 180–220 g (Moellegard Breeding Center, Denmark) fed a standard pellet diet and water ad libitum were used in this study. They were anesthetized by Hypnorm Vet® (AB Leo, Helsingborg, Sweden, 0.5 ml/kg body weight) and diazepam (2.5 mg/kg body weight) administered intramuscularly. Hypnorm Vet® is a combination of the narcotic analgesic fentanyl citrate (0.315 mg/ml) and the tranquilizer fluanisone (10 mg/ml). All experimental procedures were approved by the local Animal Ethics Committee.

To prepare chylomicrons, the thoracic duct of an anesthetized 250-g rat was cannulated in the morning (29). At the same time a cannula was inserted in the stomach. After the operation, 5% glucose, 0.85% NaCl, and 0.05% KCl were infused intragastrically at a rate of 2 ml/h. The rat was also allowed to drink ad libitum. The following morning 1 ml of the labeled LLL emulsion (100 mg TG, 50 μCi [9,10-³H]triolein) was administered via the gastric cannula. White lymph appeared in the thoracic duct tubing within half an hour. Lymph was collected for 5 h (ca 15 ml) in sterile tubes containing gentamicin (Garamycina®, Schering Corporation USA, Kenilworth, NJ) to a final concentration of 0.5 mg/ml. The lymph was defibrinated and then centrifuged at 3000 rpm for 10 min at 4°C to remove white blood cells. The lymph was then layered under 0.9% NaCl with 0.5 mg/ml of gentamicin, and centrifuged in a Beckman SW 50 rotor at 30,000 rpm for 30 min at 4°C (30). The top layer was

isolated by slicing the tube and the chylomicrons were resuspended in the saline/gentamicin buffer. The TG fraction contained 98% of the label according to TLC analysis on Silica G gel plates developed in hexane–diethyl ether–formic acid 80:20:2 (v/v/v).

The experiments were usually started in the morning (9–11 AM). Fasted rats had been without food since noon the previous day. The animals were anesthetized and emulsion was injected into the exposed left jugular vein. Blood samples (~0.1 ml) were withdrawn from the right jugular vein at 1, 2, 3, 4, 5, 7, 10, 13, 16, and 20 min into heparinized tubes and kept on ice until extraction of lipids. It should be noted that whole blood was used; no centrifugation was involved, to avoid the risk of losing emulsion. At 20 min the rats were killed by cervical dislocation. Immediately thereafter the liver, lung, heart, spleen, a piece of the epididymal fat pad, m. soleus, and a piece of m. gastrocnemius were dissected out, weighed, and frozen. M. soleus and m. gastrocnemius were chosen as well-defined examples of red and white muscles, respectively. Tissue lipids were later extracted and their radioactivity was determined (see below). The rats used to study oxidation of [³H]oleic acid-labeled TG were treated in the same way, except that no blood or tissue samples were taken and the whole carcass was put into saponification mix (see below). In the experiments with unanesthetized rats, injection was in a lateral tail vein.

Blood volumes were determined in separate experiments on corresponding rats using ^{51}Cr-labeled red blood cells (31). A mean value of 5.5% of the body weight was used for calculations on the present data.

Fifty μl of each blood sample was extracted in 2 ml of isopropanol–heptane–1 M H_2SO_4 40:48:1 (v/v) and the phases were separated by addition of 0.5 ml water (28). Tissues were homogenized and extracted in chloroform–methanol 2:1 (v/v) (32) and the phases were separated by addition of 0.4 vol of 2% KH_2PO_4. The lipid extracts were transferred to scintillation vials, scintillation liquid was added, and the radioactivity was measured in an LKB Wallac 1214 RackBeta scintillation counter (LKB-Pharmacia, Uppsala, Sweden).

Total oxidation of fatty acids was estimated as the difference between what was injected and remaining lipid-soluble radioactivity determined by digestion of the carcass in a mixture of 100 g potassium hydroxide, 100 ml distilled water, and 300 ml ethanol (95%) (33). The carcass was allowed to dissolve for 2 days at room temperature, then 50% ethanol was added to a total volume of 1000 ml. An aliquot of the digest was made acidic by addition of HCl (indicator: thymol blue, final pH <1.2) and heptane was added for extraction of fatty acids. An aliquot of the heptane was put in a scintillation vial, scintillation liquid was added, and radioactivity was measured.

Calculations

The fractional catabolic rates (FCR) given in Table 4 were determined using the SAAM30/CONSAM program (34). The equation $N = N_0 e^{-kt}$ described all data.

Statistical analysis

The two-tailed Student's t-test was used. Differences were considered significant when P was <0.05. In some experiments a one-way ANOVA and Neuman-Keuls test ($\alpha < 0.05$) were used for multiple comparisons (35). The NCSS program (Number Cruncher Statistical System version 5.01, Kaysville, UT) was used for the statistical calculations. Data are given as mean $\pm$ SEM.
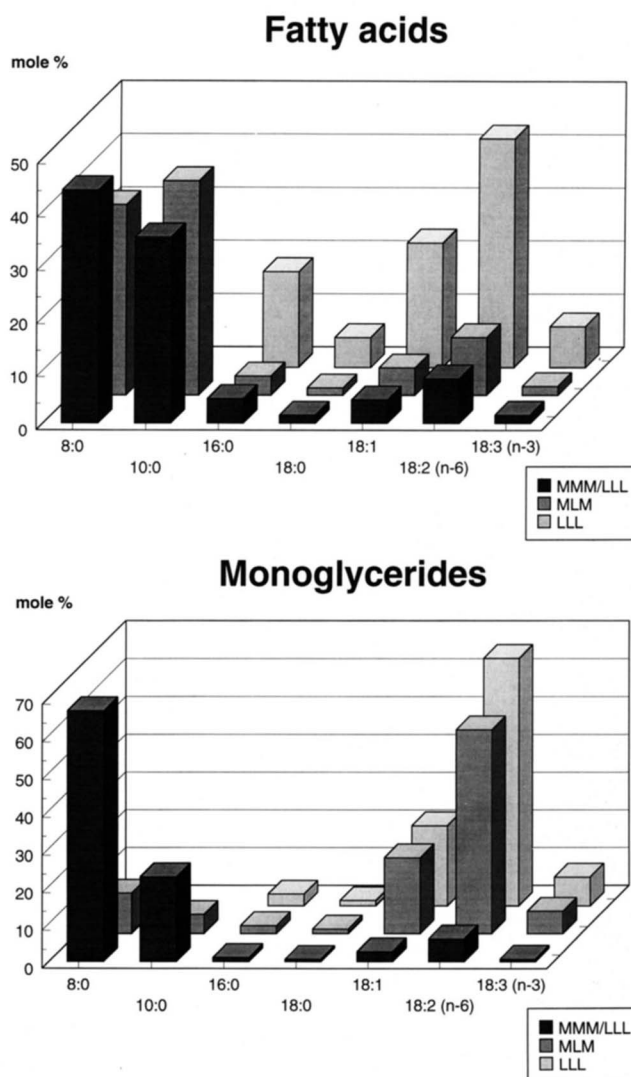

Fig. 1. Pattern of fatty acids (upper panel) and monoglycerides (lower panel) released during in vitro lipolysis of the emulsions. The three different emulsions, LLL, MMM/LLL, and MLM, were hydrolyzed in vitro by lipoprotein lipase and the products of lipolysis were analyzed according to the procedures described in methods.

RESULTS

Product patterns during in vitro lipolysis

In these experiments the emulsions were incubated with LPL under conditions yielding about 5% lipolysis in 10 min at pH 7.4 and 25°C. The main products were fatty acids and monoglycerides. Diacylglycerols were minor components in the reaction mix. From **Fig. 1** it can be seen that mainly medium-chain fatty acids were formed from the MMM/LLL and the MLM emulsions, whereas LLL yielded a mixture of long-chain fatty acids. The release of fatty acids from MMM/LLL and MLM was similar.

In contrast to the similar pattern of fatty acids released, there was a striking difference in the monoglycerides formed from MLM and MMM/LLL (Fig. 1, lower panel). In the mixed emulsion, the MMM TG were hydrolyzed first, leading to a majority of monoglycerides containing medium-chain fatty acids. In the MLM emulsion, the monoglycerides contained mainly long-chain fatty acids, reflecting the structure of the TG and the positional specificity of the lipase.

We then studied the release of labeled fatty acids from the three emulsions used for the in vivo experiments. For the LLL emulsion [³H]oleic acid (by measurement of radioactivity from the labeled triolein incorporated in the emulsion) and total fatty acids (by titration) were released at the same relative rates, within experimental error. For this, the rates were expressed as fractions of what was contained in the emulsions, e.g., radioactivity released as fraction of total radioactivity. For the MMM/LLL emulsion, [³H]oleic acid was initially released at about 25% of the rate for total fatty acids. This reflects the preferential hydrolysis of MMM TG in such mixtures of MMM and LLL (8). For the MLM emulsion, the rate for release of [³H]oleic acid was only about 7% of the rate for total fatty acid release. This reflects the 1,3-positional specificity of

lipoprotein lipase (10–12), in concert with the studies where the released fatty acids were analyzed by gas–liquid chromatography (Fig. 1 and **Table 3**). Therefore, in MLM the long-chain fatty acid is more likely to remain in a monoglyceride than in MMM/LLL.

Clearance from blood

In the first experiment, anesthetized rats were given 20 µl emulsion, corresponding to 4 mg TG. This dose was chosen as the least amount that gave sufficient radioactivity for convenient measurement of plasma and tissue radioactivity. The experiment was done in fed and in overnight-fasted rats. **Figure 2** shows disappearance curves for the three emulsions in the fed rats. In general the curves indicate an exponential decay, i.e., first order kinetics. We therefore calculated fractional catabolic rates (FCR) using data from 3–13 min. The derived FCRs are given in **Table 4.** Comparing fed and fasted rats, the FCR for LLL was significantly higher in the fasted group, whereas for MLM and for MMM/LLL there were no statistically significant differences between the nutritional states. Comparing the three emulsions, there was no significant difference between the FCRs for MLM and MMM/LLL in either nutritional state. The FCR values were lower for LLL than for the MLM or MMM/LLL emulsions, but the difference reached statistical significance only in the fed rats.

In a separate experiment we followed the clearance of chylomicrons (4 mg TG, fasted rats, data not shown). These data also followed first order kinetics and gave an FCR of 0.151 ± 0.016 min^{-1}. Hence, labeled triolein in the LLL emulsion was cleared at a rate similar to labeled oleic acid in the chylomicron TG, with half-lives of 4.2 min compared to 4.6 min.

We then studied how the rate of TG clearance changed with the amount of emulsion injected, using bolus doses of 4–64 mg TG per rat (**Fig. 3**). The observed rates were

TABLE 3.   Fatty acid composition of di- and triglycerides after in vitro lipolysis

Fatty Acid	Diglycerides			Triglycerides		
	LLL	MMM/LLL	MLM	LLL	MMM/LLL	MLM
		mol %			*mol %*	
8:0		36.0	20.6		26.8	36.2
10:0		33.8	13.6		16.4	19.5
16:0	9.0	2.9	2.2	11.2	6.4	1.7
18:0	3.2	1.0	0.8	3.7	2.1	0.5
18:1	24.2	7.6	16.2	26.0	14.9	10.7
18:2	55.3	16.2	41.7	51.2	29.0	27.8
18:3	7.1	1.9	4.7	7.9	4.4	3.4

Data are for the same experiments as in Fig. 1. The analyses were done as described in Methods by enzymatic digestion, separation of products by thin-layer chromatography, followed by gas chromatographic analysis of the fatty acids in each fraction.
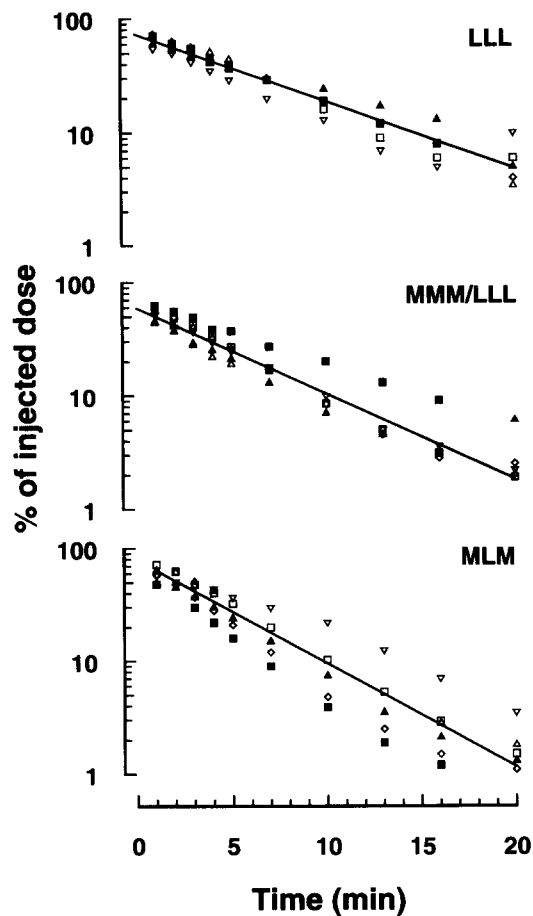
Fig. 2. Clearance of radioactivity from blood after injection of the emulsions in trace dose. Anesthetized rats were given emulsion corresponding to 4 mg TG, and serial blood samples were taken. Each type of symbol denotes data from one rat to show that the variability in each timepoint is due to different FCRs for different rats. The line illustrates the mean FCR for the group (Table 4).

in the range 0.020–1.3 μmol·ml^{-1}·min^{-1} and 0.035–1.24 μmol·ml^{-1}·min^{-1} for MMM/LLL and MLM, respectively, compared to 0.016–0.53 μmol·ml^{-1}·min^{-1} for LLL. The rates were higher for the two medium chain

TABLE 4. Fractional catabolic rates for clearing of the labeled moiety in emulsions when injected in trace dose

	Fed (min^{-1})	Fasted (min^{-1})
LLL	0.142 ± 0.007	0.167 ± 0.013[a]
Mix (MMM/[³H]-LLL)	0.191 ± 0.013[b]	0.195 ± 0.008
M-[³H]L-M	0.211 ± 0.025[c]	0.187 ± 0.022

Rats were injected with emulsion corresponding to 4 mg TG. Data for fed rats are the same as in Fig. 2. Slopes for the disappearance of label were calculated from data for 3–13 min, assuming first order kinetics using proportional weighing. Values are expressed as fractional catabolic rate (min^{-1}). Mean ± SEM for six rats in each group.
[a]$P < 0.05$ fed vs. fasted for the same emulsion (Student's t test).
[b]$P < 0.01$, [c]$P < 0.05$ vs. LLL emulsion in the same nutritional state (Student's t test).

fatty acid containing emulsions at all TG concentrations studied. There was no evidence that the clearing process could be saturated at the emulsion doses given.

Oxidation

Figure 4 shows the oxidation of the labeled oleic acid. Analysis of variance showed significant effects ($F<0.001$) of nutritional state (higher oxidation in fasted rats,) and of anesthesia (higher oxidation in unanesthetized rats). Comparing MMM/LLL and MLM, the ANOVA showed that significantly more labeled oleic acid was oxidized from MLM ($F<0.05$). This difference was small for anesthetized rats, but in the unanesthetized rats the difference was substantial. Sixty percent was oxidized from MLM compared to 41% from MMM/LLL.
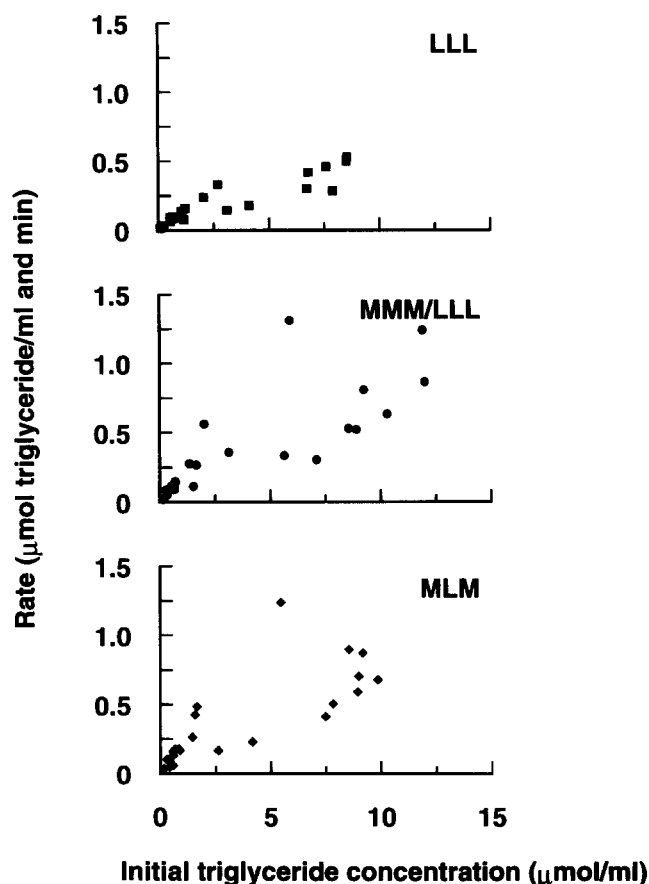

Fig. 3. Comparison of the initial rate of clearance to the initial TG concentration in vivo. Data are from experiments with radioactive LLL, MMM/LLL, and MLM emulsions and represent clearing of the labeled component. Anesthetized rats were injected with different doses of the emulsions corresponding to 4–64 mg TG. The rate of clearing was estimated from the earliest time points. These data are plotted against the initial TG concentration (calculated from radioactivity in blood at 1 min after injection).
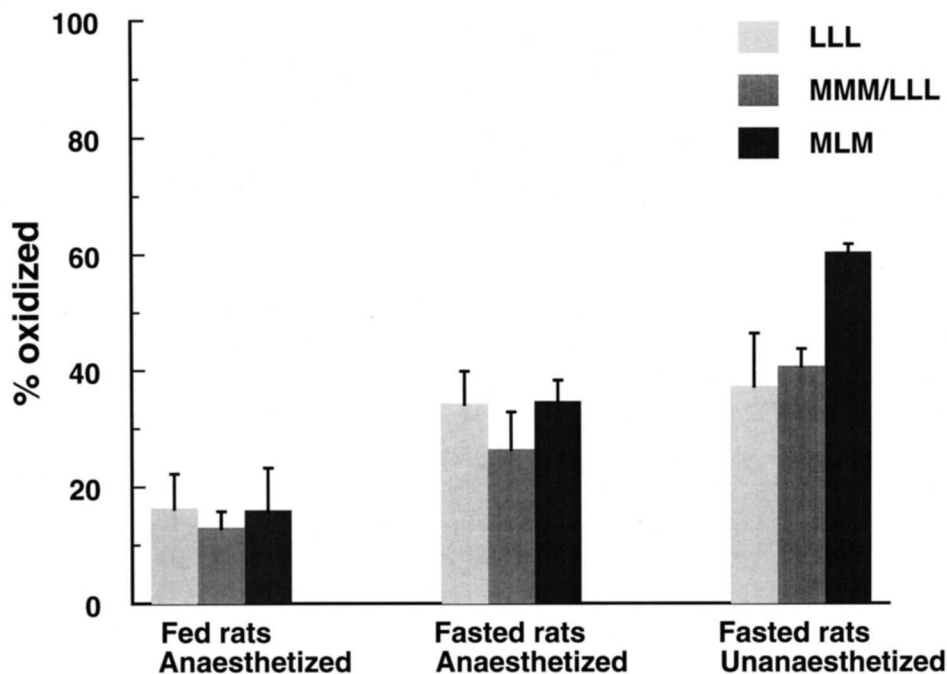
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Fig. 4. Oxidation of oleic acid from the different emulsions. Anesthetized and unanesthetized rats were given emulsion equal to 4 mg TG. LLL (light gray), MMM/LLL (gray), and MLM (dark gray). The oxidation of oleic acid was calculated from the recovery of label after digestion of the entire carcass in alkaline ethanol followed by extraction of the fatty acids. Using analysis of variance for all three emulsions, the metabolic state of the rat (fed or fasted) and anesthesia significantly affected the oxidation of labeled fatty acids. The type of emulsion had only a marginal effect on the oxidation. When only the two medium-chain emulsions were compared, ANOVA revealed a significant difference, more labeled oleic acid was oxidized from MLM.

Tissue distribution of labeled lipids

Most of these experiments were in anesthetized rats (**Fig. 5**), and included studies on the effects of nutritional state (compare panel A to panel B), and of emulsion dose (compare panel B to C). For comparison, a group of fasted rats was given labeled chylomicrons (panel A). Tissue radioactivity was determined 20 min after injection of the emulsions/chylomicrons. At this time more than 91% of the radioactivity had been removed from plasma, except in the rats given the high dose of the LLL emulsion (panel C).

Comparing first the results for the emulsions to those for chylomicrons (panel A), the major differences were more label in blood (2.5–3.3% compared to 0.69%), liver (21–28% compared to 15%), and spleen (3.1–5.5% compared to 1.2%) in the rats given emulsions. The higher uptake in spleen suggests that more of the emulsion was cleared by particle uptake in the reticuloendothelial system.

In the fasted rats given 4 mg TG (panel A), there were no major differences among the three emulsions, except for a higher uptake of MLM compared to the other emulsions in the epididymal fat pad. Nutritional state had the expected effect in the rats given the LLL emulsion: several-fold more label in adipose tissue and significantly less label in heart in the fed state. The MMM/LLL and MLM emulsions did not follow this pattern. Radioactivity in heart was as high or higher in the fed state, and the nutritional effect in adipose tissue was blunted. In the other tissues the effects of nutritional state were small for all emulsions, and in most cases not statistically significant.

Fed rats were also given a larger dose of emulsion (64 mg TG, panel C). Major differences in tissue distribution compared to the 4 mg dose were higher radioactivities left in blood for all three emulsions, lower radioactivities in liver for all three emulsions, and lower radioactivities in heart for the MMM/LLL and MLM emulsions. The tendency in the 4 mg rats for lower uptake of MLM compared to MMM/LLL in the spleen became significant when 64 mg TG was given ($P < 0.05$). With the high dose there was no significant difference in heart radioactivity between the medium chain containing emulsions and the LLL emulsion, in contrast to the findings with the lower dose.

The experiments were then repeated in unanesthetized rats (**Fig. 6**). These rats oxidized a larger fraction of the injected label (see above, Fig. 4) presumably because of higher physical activity and differences in tissue perfusion. In general, the tissue radioactivities were lower than in the anesthetized rats (compare Figs. 5 and 6). Significant differences between the emulsions were found only for liver radioactivity which was lower for MMM/LLL compared to the LLL emulsion at both doses and compared to the MLM emulsion at the higher dose.

DISCUSSION

The starting point for this research was a hypothesis that the positional specificity of LPL should lead to differences in metabolism of a long-chain fatty acid in the
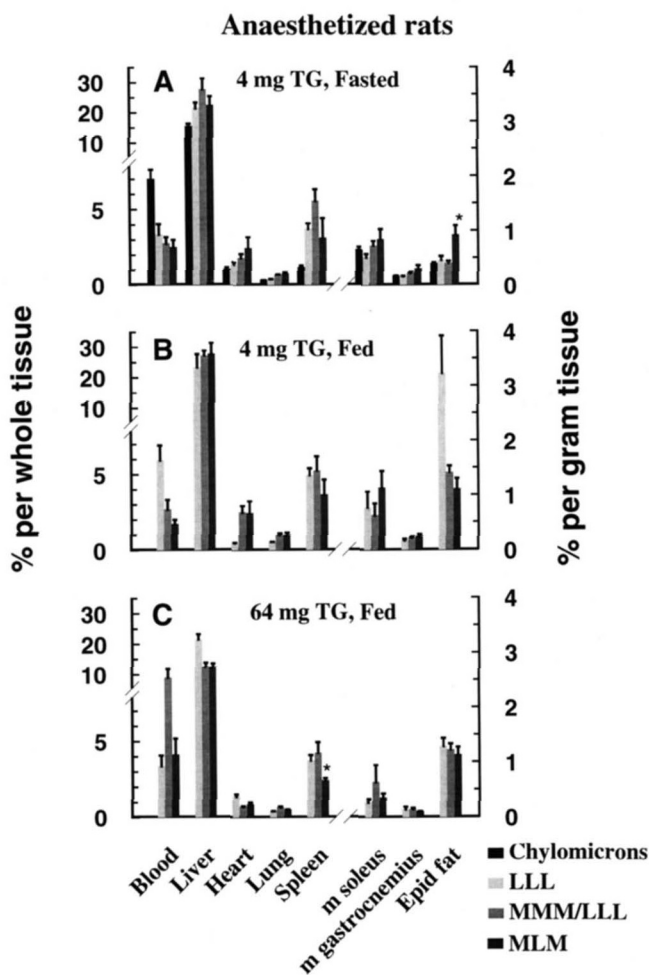
Anaesthetized rats

Fig. 5. Tissue distribution of [^{3}H]oleic acid from the emulsions in anesthetized rats. Anesthetized rats were injected with ^{3}H-labeled TG emulsions. The tissues were rapidly excised 20 min after injection, rinsed in saline, and blotted dry. After weighing, the tissue was frozen. Lipids were extracted in chloroform–methanol 2:1. Values are expressed as percent of injected radioactivity per organ (blood, liver, heart, lung, and spleen) or per gram tissue (m. soleus, m. gastrocnemius, and epididymal fat); six rats in each group. Chylomicrons, black; LLL emulsion, light gray; MMM/LLL, gray; MLM, dark gray. Panel A shows tissue distribution in fasted rats that received 4 mg TG, panel B the tissue distribution in fed rats given 4 mg TG, and panel C the tissue distribution in fed rats that received 64 mg TG. The • indicates significant differences between MMM/LLL and MLM as determined by Student's t-test ($P < 0.05$). Using ANOVA to compare the effect of different emulsions over the three groups, the following differences were found to be significant: blood (LLL vs. MMM/LLL, LLL vs. MLM), lung (LLL vs. MMM/LLL, LLL vs. MLM), and m. gastrocnemius (LLL vs. MMM/LLL).

2-position of TG, compared to one in the 1,3-position. To study this we used three emulsions, LLL with only long-chain TG, MMM/LLL with a physical mixture of MMM and LLL TG, and MLM containing bioengineered TG with medium-chain fatty acids in the 1,3-positions and long-chain fatty acids in the 2-position. The in vitro studies showed the expected large differences in product

patterns during LPL-mediated lipolysis, but the in vivo studies showed only marginal differences in clearance, tissue distribution, or oxidation. In most of the studies we used a trace dose, so that the medium-chain fatty acid should not affect general metabolism. During infusion of these emulsions in clinical situations, it is likely that the medium-chain fatty acids cover a large proportion of the energy needs and substantially suppress immediate oxidation of the long-chain fatty acids. In the present study we were not concerned with these metabolic aspects, but wanted to look at the effects of molecular structure.

During in vitro lipolysis of the MMM/LLL emulsion, the MMM component was preferentially cleaved. This is in accord with previous studies, and is a consequence of preferential partitioning of MMM TG to the particle surface (8). Yet, radioactive triolein in this emulsion was cleared at least as fast as in the LLL emulsion. The clearing was monoexponential even from the earliest time points. The MLM emulsion behaved in a similar manner. In vitro data suggest that initially almost all LPL-mediated hydrolysis is of medium chain fatty acids in the 1,3 position. Still the long-chain fatty acid label in the MLM emulsion is cleared at least as fast as in the LLL controls. It is difficult to reconcile this with an obligatory sequence: LPL-mediated hydrolysis of TG → formation of TG-depleted remnants → particle removal of the remnants. Rather, the finding implies substantial removal of particles at early stages of, or even before, lipolysis. These considerations cast serious doubt on the hypothesis that clearing of the emulsion was dominated by LPL-mediated lipolysis. An alternative is removal as particles, but this cannot be directly addressed here as no core marker, e.g., cholesteryl ethers, was present in the particles.

A general finding was that oxidation of radioactive oleic acid was similar in spite of being incorporated into rather different TG molecules and mixed or not with medium-chain fatty acids. For all three emulsions more oleic acid was oxidized in fasted compared to fed rats. Hence, the nutritional state had the expected effect. Analysis of variance demonstrated a significant difference between MMM/LLL and MLM. More labeled oleic acid was oxidized from the MLM emulsion. The difference was small in the anesthetized rats but was quite marked in the un-anesthetized fasted rats, the group where overall fatty acid oxidation was highest. This indicates that under conditions of accelerated fatty acid oxidation, MLM and the monoglycerides derived from it were most readily utilized by the tissues.

Uptake of chylomicron fatty acids into adipose tissue varies with the nutritional state (36–38). The LLL emulsion yielded results similar to those expected for chylomicrons. In fed rats 3.2% of the injected radioactivity was found per gram of epididymal adipose tissue, probably corresponding to deposition of about one-third of the emulsion fatty acids in the entire adipose tissue. In
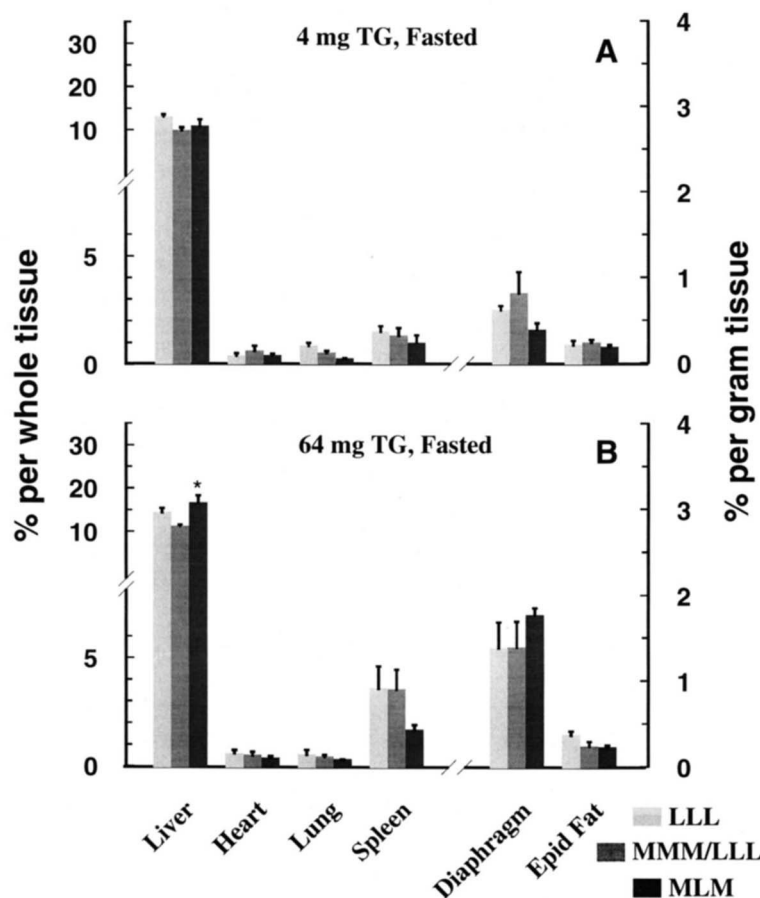
Unanaesthetized rats

Fig. 6. Tissue distribution of [³H]oleic acid from the emulsions in unanesthetized rats. Unanesthetized, fasted rats were injected with ³H-labeled TG emulsions in a tail vein. Twenty min after injection the rats were decapitated and the amount of [³H]lipid label in each organ was determined after extraction in chloroform–methanol 2:1. Panel A shows the tissue distribution after 4 mg TG and panel B after 64 mg TG. Light gray bars indicate LLL emulsion, gray MMM/LLL, and dark gray MLM emulsion. Values are expressed as percent of injected radioactivity per organ (liver, heart, lung, and spleen) or per gram tissue (diaphragm and epididymal fat). The * indicates significant differences between MMM/LLL and MLM as determined by Student's t-test ($P < 0.05$). Using ANOVA to compare the effect of different emulsions over the two groups the following differences were found to be significant: liver (LLL vs. MMM/LLL, MMM/LLL vs. MLM) and lung (LLL vs. MLM). The effect of dose was significant in liver, spleen, and epididymal fat.

fasted rats, much less, only 0.4% per gram, was found in the epididymal adipose tissue. Uptake in adipose tissue in the fed rats was less than half as high for the mixed and the structured emulsions, indicating that a lower fraction of long-chain fatty acids from these emulsions is deposited in fat stores.

Another major site of chylomicron metabolism is muscle (39, 40). Here, tissue radioactivity does not give a reliable estimate of total uptake, as some fatty acids are immediately oxidized. Radioactivity in heart was higher in rats given the mixed or structured emulsions. There was a tendency to higher uptake also in the red muscle studied, m. soleus, but this did not reach statistical significance. In the heart, the difference was most marked in fed rats where values for the mixed and the structured emulsions were more than 5 times higher compared to the LLL emulsion. This suggests that a larger fraction of lipids from these emulsions is directed to heart. Another possibility is that when emulsion TG are hydrolyzed in

the heart, oxidation of the medium chain fatty acids covers energy needs and the long-chain fatty acids tend to become esterified.

The third major destination for chylomicron fatty acids is the liver. Much of this uptake is supposed to occur with remnant particles (41, 42), but there is a substantial contribution from radioactive fatty acids recirculated in the plasma FFA pool (43, 44). We cannot directly evaluate the metabolism of remnant particles here, as we did not have any core marker. The results indicate, however, no major differences among the three emulsions, or between fed and fasted rats. In all groups the uptake was around 25%.

These studies demonstrate differences in how TG long-chain fatty acids are cleared and handled when administered in the present three emulsions. Further studies will be needed to better understand the origin of these differences and to evaluate what further differences are imposed by metabolic adaptations induced by larger doses of emulsions, and/or by disease states. ▪

REFERENCES

1. Bach, A. C., A. Frey, and O. Lutz. 1989. Clinical and experimental effects of medium chain-triglyceride-based fat emulsions—a review. *Clin. Nutr.* **8:** 223–235.
2. Lutz, O., T. Lave, A. Frey, Z. Meraihi, and A. C. Bach. 1989. Activities of lipoprotein lipase and hepatic lipase on long- and medium-chain triglyceride emulsions used in parenteral nutrition. *Metabolism.* **38:** 507–513.
3. Carpentier, Y. A., M. Richelle, D. Haumont, and R. J. Deckelbaum. 1990. New developments in fat emulsions. *Proc. Nutr. Soc.* **49:** 375–380.
4. Carpentier, Y. A., A. Van Gossum, D. Y. Dubois, and R. J. Deckelbaum. 1993. Lipid metabolism in parenteral nutrition. *In* Clinical Nutrition - Parenteral Nutrition. Rombeau and Caldwell, editors. W. B. Saunders Company, Philadelphia. 35–74.
5. Miles, J. M. 1991. Intravenous fat emulsions in nutritional support. *Curr. Opin. Gastroenterol.* **7:** 306–311.
6. Morgan, J. B., and I. Z. Kovar. 1992. The low birth weight infant and parenteral nutrition. *Nutr. Res. Rev.* **5:** 115–129.
7. Oliveira, H. C., M. H. Hirata, T. G. Redgrave, and R. C. Maranhao. 1988. Competition between chylomicrons and their remnants for plasma removal: a study with artificial emulsion models of chylomicrons. *Biochim. Biophys. Acta.* **958:** 211–217.
8. Deckelbaum, R. J., J. A. Hamilton, A. Moser, G. Bengtsson-Olivecrona, E. Butbul, Y. A. Carpentier, A. Gutman, and T. Olivecrona. 1990. Medium-chain vs. long-chain triacylglycerol emulsion hydrolysis by lipoprotein lipase and hepatic lipase: implications for the mechanism of lipase action. *Biochemistry.* **29:** 1136–1142.
9. Redgrave, T. G., and R. C. Maranhao. 1985. Metabolism of protein-free lipid emulsion models of chylomicrons in rats. *Biochim. Biophys. Acta.* **835:** 104–112.
10. Nilsson-Ehle, P., T. Egelrud, P. Belfrage, T. Olivecrona, and B. Borgström. 1973. Positional specificity of purified milk lipoprotein lipase. *J. Biol. Chem.* **248:** 6734–6737.
11. Morley, N., and A. Kuksis. 1972. Positional specificity of lipoprotein lipase. *J. Biol. Chem.* **247:** 6389–6393.
12. Åkesson, B., S. Gronowitz, B. Herslöf, P. Michelsen, and T. Olivecrona. 1983. Stereospecificity of different lipases. *Lipids.* **18:** 313–318.
13. Belfrage, P., J. Elovsson, and T. Olivecrona. 1965. Radioactivity in blood and liver partial glycerides, and liver phospholipids after intravenous administration to carbohydrate-fed rats of chyle containing double labeled triglycerides. *Biochim. Biophys. Acta.* **106:** 45–55.
14. Peterson, J., B. E. Bihain, G. Bengtsson-Olivecrona, R. J. Deckelbaum, Y. A. Carpentier, and T. Olivecrona. 1990. Fatty acid control of lipoprotein lipase: a link between energy metabolism and lipid transport. *Proc. Natl. Acad. Sci. USA.* **87:** 909–913.
15. French, J. E., and B. Morris. 1957. The removal of ^{14}C-labelled chylomicron fat from the circulation in rats. *J. Physiol. (London)* **138:** 326–339.
16. Saxena, U., and I. J. Goldberg. 1990. Interaction of lipoprotein lipase with glycosaminoglycans and apolipoprotein C-II: effects of free-fatty-acids. *Biochim. Biophys. Acta.* **1043:** 161–168.
17. Bengtsson-Olivecrona, G., and T. Olivecrona. 1991. Phospholipase activity of milk lipoprotein lipase. *Methods Enzymol.* **197:** 345–356.
18. Hansen, T. T. 1989. Specific esterification with immobilised lipase. Proceedings from the International Symposium on New Aspects of Dietary Lipids. Benefits, Hazards and Use. Sept. 17-20, 1989, Gothenburg, Sweden. (Abstract)
19. Hartman, L. 1957. Glyceride synthesis by direct esterification. *J. Chem. Soc.* 3572–3575.
20. Mattson, F. H., and R. A. Volpenheim. 1962. Synthesis and properties of glycerides. *J. Lipid Res.* **3:** 281–296.
21. Wood, T. R., F. L. Jackson, A. R. Baldwin, and H. E. Longenecker. 1944. The preparation of some unsaturated fatty acid chlorides. *J. Am. Chem. Soc.* **66:** 287–289.
22. Malkin, T., and B. R. Wilson. 1949. The X-ray and thermal examination of the glycerides. Part X. Symmetrical mono-oleoyl and monoelaidoyl disaturated triglycerides. *J. Chem. Soc.* 369–372.
23. Mank, A. P. J., J. P. Ward, and D. A. van Dorp. 1976. A versatile, flexible synthesis of 1,3-diglycerides and triglycerides. *Chem. Phys. Lipids.* **16:** 107–114.
24. Thomas, A. E., III, J. E. Scharoun, and H. Ralston. 1965. Quantitative estimation of isomeric monoglycerides by thin-layer chromatography. *J. Am. Oil. Chem. Soc.* **42:** 789–792.
25. Yurkowski, M., and H. Brockerhoff. 1966. Fatty acid distribution of triglycerides determined by deacylation with methyl magnesium bromide. *Biochim. Biophys. Acta.* **125:** 55–59.
26. Morley, N. H., A. Kuksis, D. Buchnea, and J. J. Myher. 1975. Hydrolysis of diacylglycerols by lipoprotein lipase. *J. Biol. Chem.* **250:** 3414–3418.
27. Wollenberg, K. F. 1990. Quantitative high resolution ^{13}C nuclear magnetic resonance of the olefinic and carbonyl carbons of edible vegetable oils. *J. Am. Oil Chem. Soc.* **67:** 487–494.
28. Dole, V. P. 1956. A relation between non-esterified fatty acids in plasma and the metabolism of glucose. *J. Clin. Invest.* **35:** 150–154.
29. Bollman, J., J. Cain, and J. Grindlay. 1948. Techniques for the collection of lymph from the liver, small intestine, or thoracic duct of the rat. *J. Lab. Clin. Med.* **33:** 1348–1352.
30. Chevreuil, O., M. Hultin, P. :auOstergaard, and T. Olivecrona. 1993. Biphasic effects of low-molecular-weight and conventional heparins on chylomicron clearance in rats. *Arterioscler. Thromb.* **13:** 1397–1403.
31. Hultin, M., G. Bengtsson-Olivecrona, and T. Olivecrona. 1992. Release of lipoprotein lipase to plasma by triacylglycerol emulsions. Comparison to the effect of heparin. *Biochim. Biophys. Acta.* **1125:** 97–103.
32. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
33. Olivecrona, T., and P. Belfrage. 1965. Mechanism for removal of chyle triglyceride from the circulating blood as studied with [C-14] glycerol and [H-3] palmatic acid labelled chyle. *Biochim. Biophys. Acta.* **98:** 81–93.
34. Berman, M. 1979. Kinetic analysis of turnover data. *Prog. Biochem. Pharmacol.* **15:** 67–108.

35. Zivin, J. A., and J. J. Bartko. 1976. Statistics for disinterested scientists. *Life Sci.* **18:** 15–26.

36. Nestel, P. J., W. Austin, and C. Foxman. 1969. Lipoprotein lipase content and triglyceride fatty acid uptake in adipose tissue of rats of differing body weights. *J. Lipid Res.* **10:** 383–387.

37. Bragdon, J. H., and R. S. Gordon. 1958. Tissue distribution of C^{14} after the intravenous injection of labeled chylomicrons and unesterified fatty acids in the rat. *J. Clin. Invest.* **37:** 574–578.

38. Brown, D. F., and T. Olivecrona. 1966. The effect of glucose availability and utilization on chylomicron metabolism in the rat. *Acta Physiol. Scand.* **66:** 9–18.

39. Linder, C., S. S. Chernick, T. R. Fleck, and R. O. Scow. 1976. Lipoprotein lipase and uptake of chylomicron triglyceride by skeletal muscle of rats. *Am. J. Physiol.* **231:** 860–864.

40. Coppack, S. W., R. M. Fisher, G. F. Gibbons, S. M. Humphreys, M. J. McDonough, J. L. Potts, and K. N. Frayn. 1990. Postprandial substrate deposition in human forearm and adipose tissues in vivo. *Clin. Sci.* **79:** 339–348.

41. Mahley, R. W., and M. M. Hussain. 1991. Chylomicron and chylomicron remnant catabolism. *Curr. Opin. Lipidol.* **2:** 170–176.

42. Connelly, P. W., G. F. Maguire, M. Lee, and J. A. Little. 1990. Plasma lipoproteins in familial hepatic lipase deficiency. *Arteriosclerosis.* **10:** 40–48.

43. Olivecrona, T. 1962. The metabolism of 1-C[14]-palmitic acid in the rat. *Acta Physiol. Scand.* **54:** 295–305.

44. Quarfordt, S. H., E. DeFaria, B. A. Landis, R. R. Bollinger, and Y. Yamaguchi. 1991. Transport of free fatty acid and triglyceride in anhepatic rats. *Hepatology.* **14:** 911–919.