Effect of dietary fat composition on the metabolism of triacylglycerol-rich plasma lipoproteins in the postprandial phase in meal-fed rats

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Abstract Rats conditioned to eating fixed-size meals (meals at 7 AM and 7 PM), consuming diets rich in palm oil or sunflower seed oil, were used to study the metabolism of chylomicrons and hepatic very low density lipoproteins (VLDL) as a function of time after meal consumption. Rats fed a palm oil diet had higher serum triacylglycerol levels at 7 AM, before the meal (1.96 ± 0.25 mM vs. 1.09 ± 0.09 mM) and reached higher levels postprandially (4.32 ± 0.48 mM vs. 2.87 ± 0.18 mM) than sunflower seed oil-fed animals, due to higher levels of hepatic VLDL (at 7 AM) and higher levels of chylomicrons *and* hepatic VLDL (in the postprandial phase). These differences in serum triacylglycerol concentrations between the diets tested were found not to be due to differences in hepatic VLDL triacylglycerol secretion (similar rate for both dietary groups and not very much affected by meal consumption) or chylomicron triacylglycerol secretion (similar response profiles on both diets), pointing towards differences in plasma triacylglycerol catabolism. Subsequent double-label studies on triacylglycerol catabolism of chylomicrons from palm oil- and sunflower seed oil-fed animals in chow-fed recipients showed that palm oil triacylglycerol is catabolized slower than sunflower seed oil triacylglycerol. Furthermore, activities of postheparin plasma lipoprotein lipase tended to be higher in sunflower seed oil-fed animals. From these data we conclude that the relative hypertriglyceridemia found in palm oil-fed animals is due to less efficient catabolism and not to increased synthesis of plasma triacylglycerol. — **Groot, P. H. E., B. C. J. de Boer, E. Haddeman, U. M. T. Houtsmuller, and W. C. Hülsmann.** Effect of dietary fat composition on the metabolism of triacylglycerol-rich plasma lipoproteins in the postprandial phase in meal-fed rats. *J. Lipid Res.* 1988. **29:** 541–551.

Supplementary key words palm oil • sunflower seed oil • chylomicrons • lipoprotein lipase

Although dietary effects on plasma lipoprotein metabolism have been studied extensively, relatively little attention has been paid to the effects of diet on plasma lipoprotein metabolism in the postprandial phase. Zilversmit (1) has stressed the relevance of those studies as men and women, in an affluent society, spend the larger part of each day in the fed state. The postprandial phase is characterized by the presence in plasma of triacylglycerol-rich lipoproteins of intestinal origin (chylomicrons, chylomicron remnants). Several lines of evidence have indicated that chylomicron remnants may play a role in atherogenesis (1–6), so more detailed information about the factors that determine their plasma residence time is needed. Furthermore, the postprandial metabolism of hepatic VLDL is of interest. During this period there is a flow of dietary constituents from gut to the liver that may affect the rate of VLDL synthesis. In addition, hormonal responses may have effects on both synthesis and catabolism of hepatic VLDL. In the present study we have investigated the metabolism of triacylglycerol-rich plasma lipoproteins as a function of the time after meal consumption in rats trained to eat meals containing either palm oil or sunflower seed oil.

MATERIALS AND METHODS

Animals and diets

Three-week-old, male SPF Wistar rats (Cpb/Wu, Central Institute for the Breeding of Laboratory animals) were divided into two groups according to body weight, omitting the upper and lower 10% of the population. Rats were individually housed and fed ad libitum with one of

Abbreviations: RBP, retinol binding protein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; SF, sunflower seed oil; PO, palm oil; PH-LPL, postheparin plasma lipoprotein lipase; P/S ratio, polyunsaturated/saturated fatty acid ratio; PUFA, polyunsaturated fatty acids.

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two experimental semisynthetic diets, containing palm oil or sunflower seed oil (30% of energy content). The composition and fatty acid composition of these diets are given in **Table 1.** After 5 weeks, a program was started to condition the animals to a meal-eating regimen. Meals of 7 g dry weight, made to a porridge by mixing with 7 g of water, were presented to the rats at 7 AM and 7 PM. Most animals (80%) adjusted to this feeding regimen in less than 1 week and those unable to do so were removed from the study. The size of the meal (7 g dry weight) was based on food consumption of rats fed the same diets ad libitum. The studies presented in the present work were performed in rats trained to meal-eating for a period of 3 weeks.

TABLE 1. Meal composition

Constituent	g per 14 g Porridge
Water	7
Sucrose	3.49
Casein	1.88
Fat (palm oil or sunflower seed oil)[a]	0.98
Cellulose	0.46
Salt mixture[b]	0.16
Vitamin mixture[b]	0.03

During the ad libitum feeding period, animals received a diet composed as shown in this table, except that water was not added. During both the ad libitum feeding and meal-feeding period, animals had free access to drinking water.

[a] Fatty acid composition as determined by gas-liquid chromatography (palm oil vs. sunflower seed oil): C14:0, 1.1% versus 0.1%; C16:0, 45% versus 7.2%; C18:0, 4.2% versus 4.0%; C18:1, 39% versus 19%; C18:2, 9.8% versus 67.5%; C18:3, 0.2%; C20:0, 0.3%; C20:1, 0.2%; C22:0, trace versus 0.6%.

[b] Details described in ref. 7.

Protocol for the measurement of serum chylomicron and hepatic VLDL concentrations and their rate of secretion into plasma

Groups of 6 rats were used for each timepoint studied (7 AM to 7 PM period, timepoints every 2 hr). All animals received last meals, supplemented with 10 μCi of [11,12(n)-^{3}H]retinol on the day of the experiment and were kept in their normal housing until the time of blood collection. At the appropriate hour, blood samples of 2 ml were collected from the tail for serum preparation. Next, the animals received a bolus of Triton WR 1339 (20% w/v in 0.9% NaCl; 2 ml/kg body weight) by penal vein injection without anesthesia and blood samples were collected after 15 min (0.5 ml) and 30 min (as the animal was killed) for serum preparation. Aliquots of serum from each rat, before and after Triton injection, were stored at −20°C in the dark for analysis of triacylglycerol, cholesterol, and radioactivity. Lipoprotein fractionations and analyses were performed in pooled serum samples (6 × 0.5 ml) immediately after the serum preparation. For each timepoint two serum pools were made up, a pre-Triton pool and a 30-min post-Triton pool. These fractions were layered on two identical columns filled with 2% agarose gel (Bio-Rad, A-50, 100–200 mesh), equilibrated with 0.9% NaCl, 10 mM Tris-HCl, 1 mM EDTA, pH 7.5. The columns were eluted with the same medium at 4°C in the dark and fractions of 1.8 ml were collected (flow rate 8 ml/hr) and analyzed for triacylglycerol, cholesterol, and radioactivity. In some experiments the label distribution was further analyzed by thin-layer chromatography after lipid extraction (8). As indicated in the Results section, ^{3}H radioactivity in the chylomicron- and VLDL-containing fractions obtained by agarose gel chromatography was nearly all in [^{3}H]retinyl esters (95–98%), while ^{3}H radioactivity associated with the serum protein peak was associated with unesterified retinol (> 97%).

Measurements of postheparin plasma lipolytic activities

Heparin (Tromboliquine, Organon, Oss, The Netherlands; 100 U/kg body weight) was injected into the penal vein in rats, using a light ether anesthesia. Ten min later, a small blood sample was collected (0.5 ml) from the tail and postheparin plasma was prepared at 4°C and frozen at −80°C. Lipoprotein lipase was determined at 37°C in the presence of 0.08 M NaCl in plasma samples preincubated with anti-rat hepatic lipase rabbit IgG (9), using an emulsion of labeled triacylglycerol stabilized with egg phosphatidylcholine, prepared by sonication in glycerol (10). Endogenous substrate in plasma was found not to affect the lipase measurements under the assay conditions employed.

Studies with mesenteric lymph

Mesenteric lymph was collected from meal-fed rats (on palm oil or sunflower seed oil diets) equipped with a mesenteric cannula (11) 2 hr after meal consumption (9 AM). For studies on the size distribution of chylomicrons in lymph, [^{3}H]retinol was added to the last meal as described above. Donor animals for lymph used in chylomicron catabolism studies received last meals mixed with 40 μCi of [9,10-^{3}H]oleic acid or 18 μCi of [1-^{14}C]oleic acid.

Chemical analysis

Triacylglycerol and cholesterol concentrations in sera and fractions obtained by agarose gel chromatography were determined by enzymatic methods (Boehringer Mannheim) using a PA 800 programmable analyzer (Vitatron, Dieren, The Netherlands) and test kits TG644200 and TC 701912. Liquid scintillation spectrometry was performed in a Packard Tri-Carb CD liquid scintillation counter using Emulsifier Scintillator 299 (Packard).

RESULTS

Postprandial changes in serum triacylglycerol concentrations

Postprandial responses in serum triacylglycerol concentrations in rats consuming a palm oil-rich or sunflower seed oil-rich diet are shown in **Fig. 1.** Rats were bled either just before their 7 AM meal (time zero) or at times after consumption of the meal as indicated in the figure. Serum triacylglycerol concentrations just before the meal were lower in sunflower seed oil-fed rats than in the palm oil-fed group. In both diets there was a steep rise in serum triacylglycerol concentration after the meal, followed by a stabilization period. Between 4 and 8 hr, a second peak in serum triacylglycerol concentration was evident, followed by a decline to pre-feeding values. At all timepoints tested, triacylglycerol concentrations in palm oil-fed rats were higher ($0.001 < P < 0.01$) than in the corresponding group fed sunflower seed oil.

Serum lipoprotein fractionation by 2% agarose gel permeation chromatography

The nature of the carriers of triacylglycerol in serum at different times after the meal was investigated further. To differentiate between triacylglycerol-rich lipoproteins of intestinal (chylomicrons and chylomicron remnants) and hepatic (VLDL) origin, intestinal lipoproteins were radiolabeled in the retinylester moiety by oral administration of [^{3}H]retinol (2, 12–14). Its validity is based on the finding that retinyl esters remain associated with the chylomicron core during metabolic processing of chylomicrons in the plasma compartment. This holds especially for rats, which lack the plasma lipid transfer activity found in other species (15). In a set of experiments designed to identify the nature of the postprandial hypertriglyceridemia, we included a defined quantity of [^{3}H]retinol in the last meals of rats (six rats per group, 2 × seven groups bled at 7 AM, 9 AM, 11 AM, 1 PM, 3 PM, 5 PM, and 7 PM, respectively) and fractionated the sera (a pool of 6 × 0.5 ml for each timepoint) by 2% agarose gel chromatography (16). **Fig. 2** shows the elution profiles of serum triacylglycerol, cholesterol, and ^{3}H radioactivity of rats fed palm oil- or sunflower seed oil-rich diets and bled at 7 AM (to illustrate the basal profile), 9 AM (to illustrate the appearance of the chylomicron peak), and 1 or 3 PM (to illustrate the elution profile at maximum plasma triacylglycerol concentration). Based on the results of triacylglycerol determinations, ^{3}H radioactivity analysis (showing that 95–98% of the ^{3}H radioactivity in fractions eluting between 40 and 120 ml represents [^{3}H]retinyl esters, while nearly all the radioactivity in the 120–140 ml eluate represents [^{3}H]retinol) and cholesterol determinations, it was concluded that the first peak in the triacylglycerol profile (40–70 ml elution volume) represents lipoproteins originating from the gut (chylomicrons and possibly chylomicron remnants) while the second peak (70–120 ml elution volume) represents mainly hepatic VLDL. Judged from the low level of radioactivity in the latter peak, contributions of small chylomicrons and chylomicron remnants to the pool of triacylglycerol associated with this second peak are probably small (see below). The apparent third peak in the triacylglycerol profile represents unesterified glycerol. The major peak in the cholesterol profile (elution volume 110–140 ml) and the late peak in the ^{3}H radioactivity profile (elution volume 120–140 ml) represent serum HDL and the retinol-RBP complex, respectively. LDL, present in rat serum in a low concentration, is eluted in the ascending limb of the major cholesterol peak.

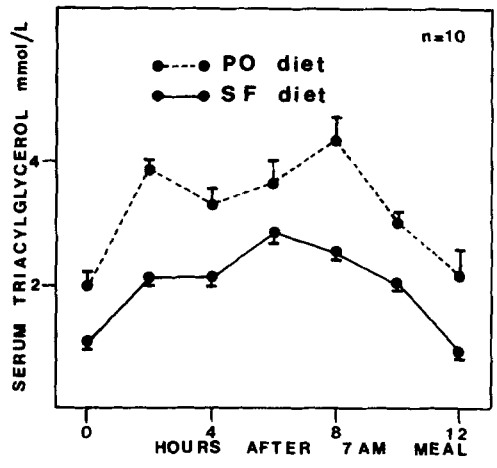

Fig. 1. Postprandial changes in serum triacylglycerol concentrations in meal-fed rats on a palm oil- or sunflower seed oil-rich diet. Each timepoint (time after the 7 AM meal) represents data from 10 animals (geometric mean ± coefficient of variation of the mean, as indicated by the bars). Different groups of rats were used for the assessment of serum triacylglycerol concentrations at all timepoints shown in the figure; (●---●) palm oil-fed rats; (●—●) sunflower seed oil-fed rats.

Analysis of mesenteric lymph of rats fed a palm oil- or sunflower seed oil-rich meal

To obtain information about the size distribution and [^{3}H]retinyl ester content of chylomicrons we collected mesenteric lymph of rats that had received a 7 AM meal supplemented with [^{3}H]retinol. Cannulation was started at 9 AM and lymph was collected between 9:30 AM and 5 PM. Aliquots of lymph from palm oil- and sunflower seed oil-fed rats were fractionated by 2% agarose gel permeation chromatography and analyzed for triacylglycerol and ^{3}H radioactivity distribution. The results of these studies are shown in **Fig. 3.** Profiles of triacylglycerol and ^{3}H radioactivity showed single peaks coinciding with the void volume of these columns and consider-

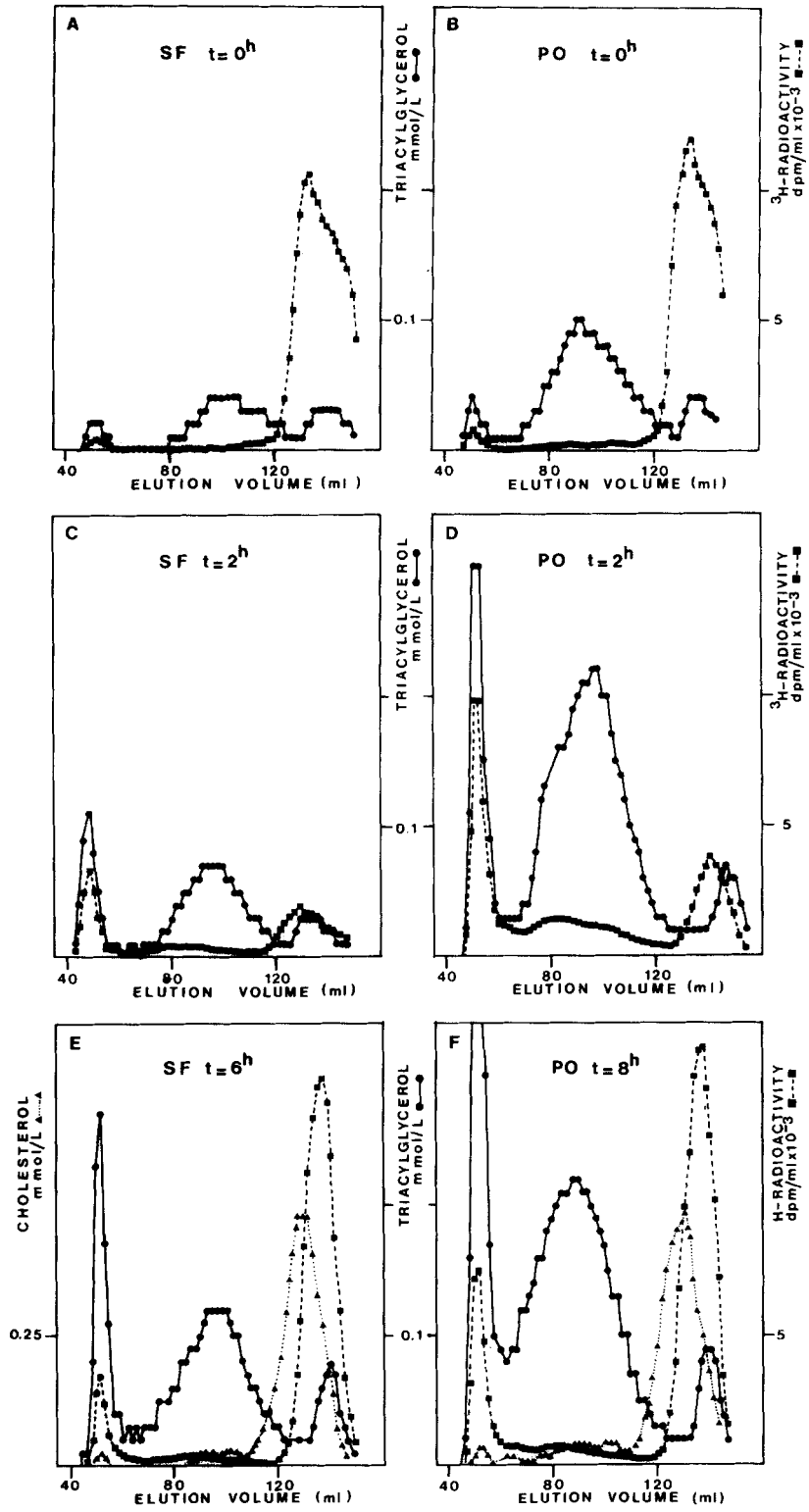

Fig. 2. Fractionation of pre- and postprandial sera of meal-fed rats by 2% agarose gel chromatography. All rats received 10 μCi of [11,12-^{3}H]retinol mixed in their last meal (for t = 0 hr: in the 7 PM meal; for t = 2 hr, 6 hr, and 8 hr: in the 7 AM meal). Rats were bled at times after 7 AM as indicated in the figure. Two percent agarose gel permeation chromatography was performed using 3 ml of a serum pool from six rats (6 × 0.5 ml). Eluted fractions were analyzed for triacylglycerol content (●—●), ^{3}H-radioactivity (■- - -■), and cholesterol (▲ · · · ▲) (for reasons of clarity only shown in the t = 6 and 8 hr sera analyses). The peak in the triacylglycerol profile at about 140 ml represents free glycerol; SF, sunflower seed oil-fed rats; PO, palm oil-fed rats. Note that the 7 AM animals received [^{3}H]retinol 12 hr earlier. Therefore, high levels of radioactivity are associated with the retinol-RBP area (elution volume 120–160 ml) in the elution profile.

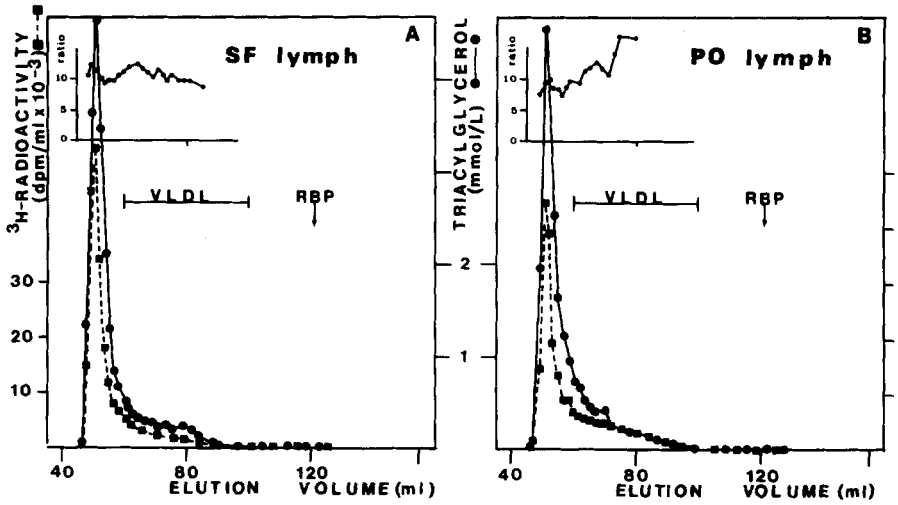

Fig. 3. Fractionation of mesenteric lymph, collected from meal-fed rats, by 2% agarose gel chromatography. Mesenteric lymph was collected from rats fed a meal containing sunflower seed oil or palm oil diet, supplemented with [^{3}H]retinol at 7 AM. Aliquots (3 ml) of (diluted) lymph, collected between 9:30 AM and 5 PM, were fractionated by 2% agarose gel chromatography and analyzed for triacylglycerol and ^{3}H radioactivity: ratio of ^{3}H radioactivity/triacylglycerol (dpm/nmol). Elution positions of VLDL and retinol binding protein (RBP), as determined with rat serum in separate runs on the same column, are indicated in the figure. For symbols see Fig. 2.

able tailing in the descending limb. It is evident that the bulk of triacylglycerol in mesenteric lymph is associated with the elution area designated as "chylomicron fraction" in the serum fractionation studies shown in Fig. 2. Only minor quantities were found in the VLDL area. Ratios of ^{3}H radioactivity and triacylglycerol were more or less constant throughout the elution profile in sunflower seed oil-fed rats but increased somewhat in the small chylomicron (VLDL) fraction in palm oil-fed rats.

In the corresponding serum fractionation studies, shown in Fig. 2, some of the [^{3}H]retinyl ester radioactivity was found in the VLDL area of the elution profile, indicating that small chylomicrons or partially delipidated chylomicrons are associated with these fractions. Using the [^{3}H]retinyl ester/triacylglycerol ratios in the chylomicron peak of each serum, one may estimate the maximal contribution of gut-derived triacylglycerol to the serum VLDL peak. These calculations revealed that 17 to 37% of VLDL-associated triacylglycerol in the sera analyzed in the present study may be of intestinal origin. However, as partially delipidated chylomicrons (having high [^{3}H]retinyl ester/triacylglycerol ratios) will also be found in this fraction and small chylomicrons may have a somewhat higher ratio to start with (see above), true contributions of the gut to the serum VLDL triacylglycerol peak will be lower. Although not entirely accurate, the contributions of the gut and liver to the serum triacylglycerol concentration can be calculated from the triacylglycerol mass associated with the first and second peak in the agarose triacylglycerol profile, respectively. However, one should keep in mind that in doing so the contribution of the gut is slightly underestimated while that of the liver is overestimated.

Estimation of the contribution of gut and liver derived triacylglycerol to the concentration of triacylglycerol in pre- and postprandial sera

A complete set of sera covering the time span 7 AM–7 PM was analyzed as shown in Fig. 2 and estimations of contributions of gut and liver to serum triacylglycerol concentrations were calculated as described above. The results of these analyses are shown in **Fig. 4.** At 7 AM just before the meal, the contribution of intestinal lipoproteins to the serum triacylglycerol concentration was low both in palm oil- and sunflower seed oil-fed rats. In response to the meal, sunflower seed oil-fed rats developed a gradual chylomicronemia peaking at 6 hr after the meal, followed by a decline to low levels at 7 PM. The postprandial chylomicronemia in palm oil-fed rats was more complex, showing peaks at 2 and 8 hr after the meal. Interesting changes in serum hepatic VLDL concentration were also observed. Both in sunflower seed oil- and palm oil-fed rats a profile was observed that followed the profile of the triacylglycerol-rich lipoproteins originating from the gut. In absolute terms the contribution of hepatic VLDL to the postprandial increase in serum triacylglycerol was even larger that that of lipoproteins of intestinal origin. Chylomicrons are removed from the circulation in a two-step process. In the first step triacylglycerol is lost by lipolysis catalyzed by lipoprotein lipase and the resulting core remnant, containing all the retinyl ester originally present in the chylomicron particle, is subsequently re-

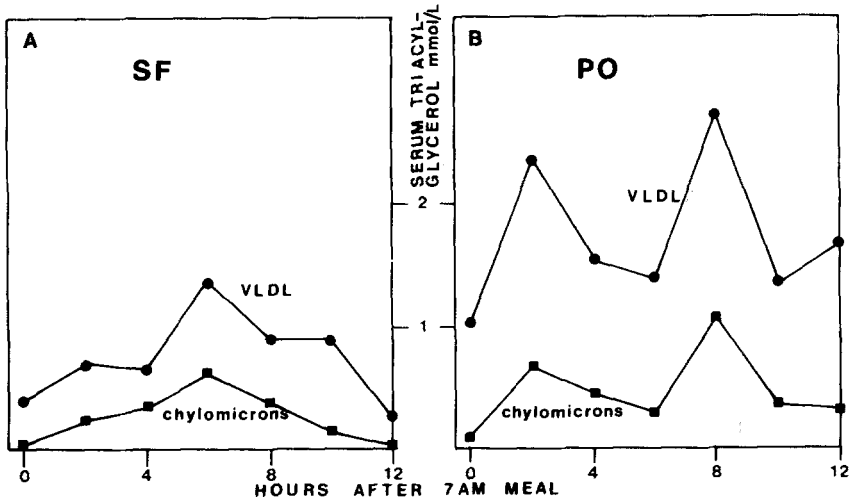

Fig. 4. Serum triacylglycerol concentrations of intestinal and hepatic origin during the postprandial phase. Sera from rats, taken at set times after their 7 AM meal as indicated in the figures, were fractionated by 2% agarose gel chromatography as indicated in Fig. 2 (for each timepoint a mixture of 0.5 ml of serum from six rats was fractionated). After determination of the elution profiles for triacylglycerol and ^{3}H radioactivity, two areas were distinguished: chylomicrons eluting between 40 and 70 ml, and VLDL eluting between 70 and 120 ml. The mass of triacylglycerol associated with both fractions was calculated and is shown in the figure; SF, sunflower seed oil-fed rats; PO, palm oil-fed rats; (●—●) triacylglycerol associated with the VLDL fraction; (■—■) triacylglycerol associated with the chylomicron fraction. For further details see the text.

moved by the liver. Close examination of the [^{3}H]retinyl ester/triacylglycerol ratio in the 40–70 ml peak of all sera analyzed by 2% agarose gel chromatography revealed that these ratios were fairly constant, both as a function of time after the meal and when palm oil- and sunflower seed oil-fed animals were compared (ratios varied between 13 and 18 × 10^{3} dpm/μmol triacylglycerol as compared to 19 × 10^{3} dpm/μmol in the meal) suggesting that the observed differences in serum triacylglycerol between palm oil- and sunflower seed oil-fed rats are not due to accumulation of chylomicron remnants in plasma. However, 2 hr after consumption of either a palm oil or sunflower seed oil meal, the ratio of [^{3}H]retinyl ester/triacylglycerol in the 40–70 ml peak was increased (30–36 × 10^{3} dpm/μmol) suggesting that at this timepoint more chylomicron remnant-like particles are present in plasma than at any of the other timepoints tested.

Determinations of plasma entrance rates (synthetic rates)

In order to investigate whether differences in serum VLDL and chylomicrons are due to differences in the rate of synthesis or rate of catabolism, a protocol was developed to measure synthetic rates. Inhibition by intravenous injection of Triton WR 1339 of VLDL triacylglycerol catabolism is well established (17–19) and it was found in preliminary experiments in this study that chylomicron catabolism is also completely blocked for periods up to at least 45 min (**Table 2**).

To estimate the plasma entrance rates of triacylglycerols in chylomicrons and hepatic VLDL in vivo, we used animals fed [^{3}H]retinol in their last meal and determined the accumulation of triacylglycerol-rich lipoproteins during a 30-min period after intravenous Triton injection. The

TABLE 2. The effect of intravenous injection of Triton WR 1339 in rats on removal from plasma of chylomicron constituents

	Time			
Serum Concentration	4 Min	19 Min	34 Min	49 Min
^{3}H-Labeled retinyl esters	100%	101 ± 8	104 ± 6	101 ± 12
^{14}C-Labeled triacylglycerol	100%	100 ± 7	103 ± 7	98 ± 9
Protein	100%	109 ± 5	109 ± 8	101 ± 7

Triton WR 1339 (20% w/v solution in 0.9% NaCl; 2 ml/kg body weight) was injected into a tail vein in unanesthetized chow-fed rats. After 2 min, intestinal lymph of a donor animal was injected (0.75 ml containing 7.4 mg of triacylglycerol/ml, labeled in the triacylglycerol and retinyl ester moieties with [1-^{14}C]palmitic acid and [11,12-^{3}H]retinol, respectively). Blood samples were taken from the tail at 4, 19, 34, and 49 min after Triton injection and serum was analyzed for ^{14}C and ^{3}H radioactivity and protein concentration. Data (mean value ± SD, n = 9) are expressed as percentage of the t = 4 min values.

accumulation of triacylglycerol was linear during this time span (data not shown). We decided to measure the accumulation during this relatively short period, as VLDL synthesis in liver may be affected by uptake by the liver of elements derived from chylomicron catabolism not available during blockade of catabolism (free fatty acids from chylomicrons; triacylglycerol from chylomicron remnants).

Post-Triton sera (pools of 6 × 0.5 ml for each timepoint) were fractionated by 2% agarose gel chromatography. A good separation between lipoproteins of intestinal origin and hepatic VLDL was obtained as illustrated by the data from the sera obtained 2 hr (plus 30 min) after the meal (**Fig. 5**). (Such data were collected at all forementioned timepoints, profiles not shown.) Once again the determinations of [^{3}H]retinyl ester radioactivity indicate that the contribution of lipoproteins of intestinal origin to the second triacylglycerol peak is rather low, and for the calculations it is assumed that the mass of triacylglycerol in this peak is solely due to hepatic VLDL. As similar data are available for sera of the same rats directly before Triton injection (the sets of data shown in Fig. 2 and used for Fig. 4), production rates of chylomicron and hepatic VLDL triacylglycerol could be estimated by subtraction. The results of the estimated rates of synthesis of chylomicron and hepatic VLDL triacylglycerol are given in **Fig. 6.** The rate of chylomicron synthesis is low just before consumption of the 7 AM meal but increased dramatically in the next 2 hr. The peak in the chylomicron production rate is followed by a stabilization period 4 to 8 hr after the meal and appears to decline to a lower level at 12 hr after meal consumption. The profiles of chylomicron production rates in animals fed palm oil and sunflower seed oil diets are similar except for an unexplained difference at the 10 hr timepoint. The time course of hepatic VLDL triacylglycerol synthesis is shown in Fig. 6B. Although minor effects on the rate of hepatic VLDL triacylglycerol production cannot be excluded from our present data, we would suggest that no major changes occur in the 7 AM to 7 PM observation period. As found for chylomicrons, the production rates of VLDL in rats fed the palm oil diet are not very much different from those found in rats fed sunflower seed oil.

Plasma triacylglycerol catabolism

As the relative hypertriglyceridemia in palm oil-fed rats could not be explained at the level of synthesis, attention was directed towards plasma triacylglycerol catabolism. To investigate the lipolytic capacity at different timepoints in the feeding cycle, lipoprotein lipase activity measurements were performed in postheparin plasma of rats fed either of the two diets studied. Although PH-LPL activities were somewhat higher in sunflower seed oil-fed animals (**Fig. 7**), no clearcut relation was observed between the timing of the hypertriglyceridemia and lipase activity (e.g., differences in PH-LPL were small midday, while differences in plasma triacylglycerol concentrations between the diets were large at that time). Therefore, the

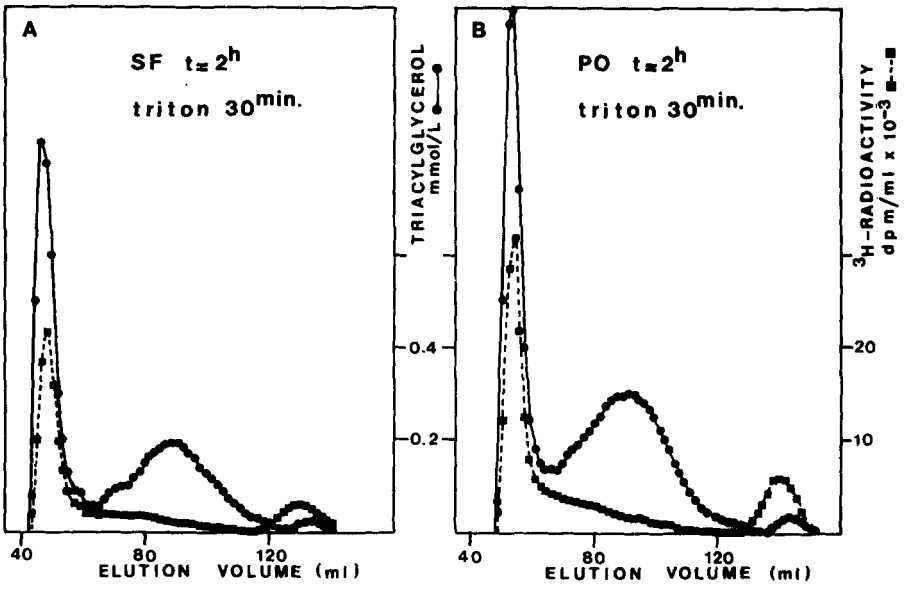

Fig. 5. Fractionation of chylomicrons and VLDL accumulated in plasma of meal-fed rats during 30 min following intravenous injection of Triton WR 1339. Sunflower seed oil (SF)- or palm oil (PO)-fed rats received a dose of Triton WR 1339 2 hr after the 7 AM meal as described in Methods and were bled 30 min later. A serum pool of 6 × 0.5 ml was fractionated by 2% agarose gel chromatography as described in Fig. 2. Serum obtained from the same rats before injection of Triton WR 1339 was analyzed by the same procedure and elution patterns of these analyses are given in Fig. 2. In comparing the pre- and post-Triton profiles, note the differences in scales. For symbols see Fig. 2.

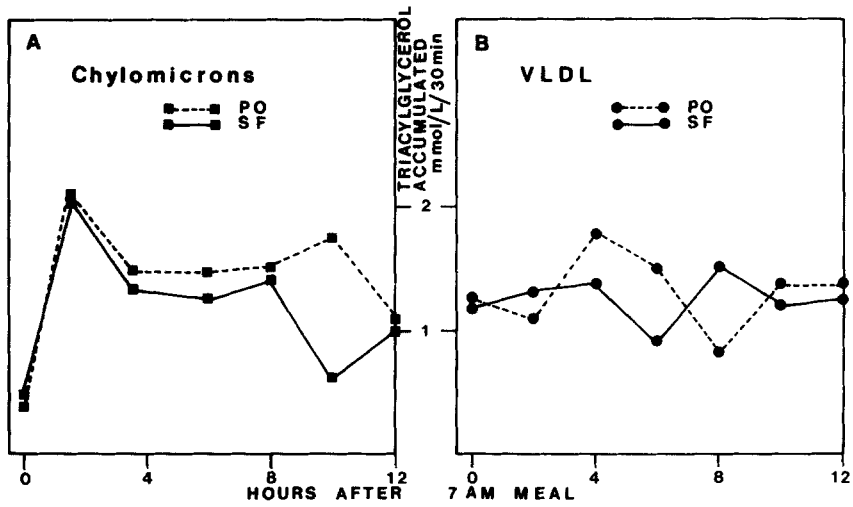

Fig. 6. Synthetic rates of chylomicron (A) and hepatic VLDL (B) triacylglycerol. Rates of chylomicron and hepatic VLDL production were calculated as the difference in triacylglycerol concentration before and 30 min after intravenous injection of Triton WR 1339. For details see Figs. 2–5.

effects of substrate type on in vivo lipolysis were investigated. For these experiments we isolated lymph from meal-fed donor rats fed either palm oil or sunflower seed oil diets. These lymph samples were labeled in vivo in their triacylglycerol moiety by adding [^{3}H]- or [^{14}C]oleic acid to the meal consumed prior to lymph collection. Lymph samples of palm oil- and sunflower seed oil-fed animals, carrying opposite labels, were mixed 1:1 based on triacylglycerol content and the catabolism of palm oil and sunflower seed oil triacylglycerol in chylomicrons was studied subsequently in chow-fed rats injected intravenously with the lymph mixture. Data from these studies are shown in **Table 3** and **Fig. 8.** It was consistently found (in all 19 animals tested, either label combination) that triacylglycerol carried on sunflower seed oil chylomicrons was catabolized faster than palm oil triacylglycerol. Data shown in Fig. 8 indicate that the half-life of sunflower seed oil triacylglycerol in plasma is about 10% shorter than that of palm oil triacylglycerol, suggesting strongly that substrate type is a major determinant for plasma triacylglycerol concentrations.

DISCUSSION

In the present work we have investigated the metabolism of plasma triacylglycerol-rich lipoproteins during the postprandial phase using meal-fed rats consuming either a palmitic and oleic acid-rich diet (palm oil) or a diet rich in linoleic acid (sunflower seed oil). The P/S ratio of palm oil resembles the one found in the fat component of the average West European diet, while sunflower seed oil was used to compose a PUFA-enriched diet. At all timepoints tested, plasma triacylglycerol concentrations in palm oil-fed rats were found to be higher than in sunflower seed oil-fed animals. Differences in postprandial plasma triacylglycerol concentrations between the two diets tested do not seem to be a direct result of differences in gastrointestinal handling of exogenous lipid. No indications were found that the more saturated fat (palm oil) was

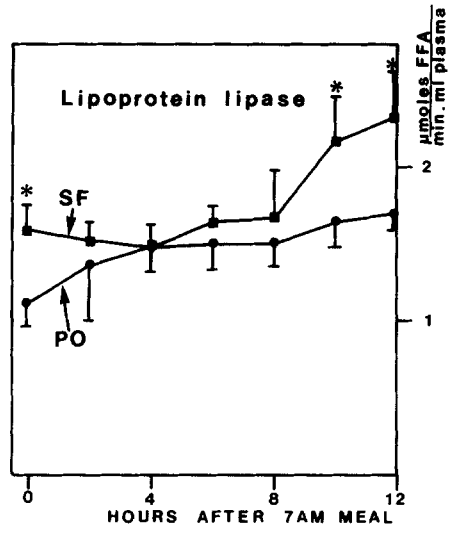

Fig. 7. Postheparin plasma lipoprotein lipase in meal-fed rats before and after a 7 AM meal. For details see Methods section; (■—■) sunflower seed oil-fed animals; (●—●) palm oil-fed animals. Each timepoint (time after 7 AM meal) represents data from six animals (mean ± SD, as indicated by the bar). Lipoprotein lipase activities decrease during the night to the level found at 7 AM (data not shown). *Statistically significant difference in lipase activity between both diets tested ($P < 0.05$).

TABLE 3. Catabolism of palm oil and sunflower seed oil lymph triacylglycerol in chow-fed recipient rats

Injected Lymph Mixture	Number of Recipients	Ratio of Plasma Triacylglycerol Radioactivity $\frac{\text{radioactivity t = 10 min}}{\text{radioactivity t = 1 min}} \times 100$	
		Palm Oil Triacylglycerol	Sunflower Seed Oil Triacylglycerol
^{3}H-Labeled palm oil + ^{14}C-labeled sunflower seed oil	12	34.6 ± 12.4	31.4 ± 12.4[a]
^{14}C-Labeled palm oil + ^{3}H-labeled sunflower seed oil	7	43.3 ± 9.0	41.9 ± 8.8[b]

Intestinal lymph was collected at room temperature from meal-fed donor rats on palm oil- or sunflower seed oil-rich diets, fed last meals supplemented with [9,10-^{3}H]oleic acid (40 μCi) or [1-^{14}C]oleic acid (18 μCi). Lymph samples collected between 2 and 5 hr after meal consumption were mixed 1:1 based on triacylglycerol content and injected intravenously (penal vein; 17.5 μmol of triacylglycerol) into chow-fed recipient animals. Blood was collected from the tail at t = 1 min (to determine 100% level) and 10 min, and sera were extracted with chloroform–methanol according to Bligh and Dyer (8). Neutral lipids were separated by TLC and associated radioactivity was determined by liquid scintillation spectrometry. Percentages of serum radioactivity associated with triacylglycerol were >95% (at t = 1 min) or >86% (at t = 10 min).

[a] Statistically significant difference between palm oil and sunflower seed oil in paired two-tailed *t*-test; $P < 0.0001$.

[b] $P < 0.01$.

digested at a slower rate as has been suggested by other studies under different conditions of fat loading (intraduodenal infusion with palmitic and linoleic acid (20) or ad libitum overnight feeding (21)). Using 2% agarose gel permeation chromatography, we demonstrated that most of the triacylglycerol in intestinal lymph, collected after meal consumption, was associated with large chylomicrons eluted at or near the exclusion volume of the column. Only a minor fraction (10–15%) was found associated with VLDL-sized chylomicrons and our data indicate that this fraction is not much different in palm oil- and sunflower seed oil-fed animals. Saturated fat feeding has been associated with an increase in VLDL-sized chylomicrons (20, 22, 23) but these findings are possibly based on artefacts due to chylomicron disintegration by cooling down lymph as recently suggested by Renner et al. (24). Although we also analyzed lymph and serum at 4°C (Renner's report appeared after this study was completed) there are no indications in our data that chylomicron disintegration had taken place in material from palm oil-fed animals.

The postprandial responses in plasma triacylglycerol concentrations in our studies tend to be biphasic, especially after palm oil meals. Such biphasic triacylglycerol responses have also been found in humans (25) and associated with increases in hepatic VLDL synthesis as a consequence of chylomicron remnant uptake (26). Assessment in this study of the contribution of gut and liver to the postprandial hypertriglyceridemia indicated that the differences between palm oil- and sunflower seed oil-fed rats are due to chylomicrons as well as hepatic VLDL and are based on differences in triacylglycerol catabolism rather than synthesis. As no marked differences were found between the two diets tested in the ratio of retinyl esters to triacylglycerol concentration in the gut-derived lipoproteins, we suggest that the higher plasma triacylglycerol concentrations in palm oil-fed rats are not due to delayed chylo-

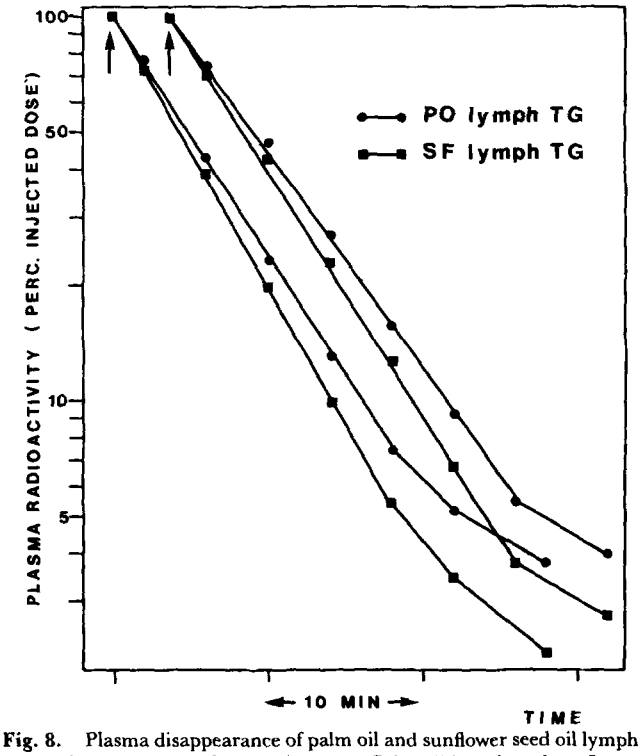

Fig. 8. Plasma disappearance of palm oil and sunflower seed oil lymph triacylglycerol in chow-fed recipient rats. Palm oil lymph and sunflower seed oil lymph labeled in the triacylglycerol moiety with [9,10-^{3}H]- and [1-^{14}C]oleic acid, respectively, were isolated as described in Table 2. A 1:1 mixture, based on triacylglycerol content, of palm oil and sunflower seed oil lymph was injected into two chow-fed recipient rats and blood was collected from the tail at t = 1 min (to determine 100% level injected dose) and t = 5, 9, 13, 17, 25, and 31 min. Data are expressed as % of initial serum radioactivity. For further details see Table 2 and the text.

micron remnant clearance but rather to a less efficient triacylglycerol hydrolysis. Relevant to this conclusion is our finding that the rates of absorption of retinol and triacylglycerol by the gut are comparable throughout the 12-hr observation period as was concluded from the constant ratio of [^{3}H]retinyl esters to triacylglycerol in lymph collected during this period (results not shown). Under our experimental conditions, Triton WR 1339 was able to block the removal from plasma of chylomicron core lipids completely (Table 2), allowing us to measure plasma entrance rates of chylomicron triacylglycerols accurately. Nagata and Zilversmit (27) recently studied the effectiveness of Triton WR 1339 to block plasma removal of chylomicron core constituents in rabbits. They found that the blockage is not complete (about 50%) but their experimental conditions were different from ours in the present study (Triton concentrations 2.5 times lower and much longer test period).

Our observation that the contribution of hepatic VLDL to the postprandial hypertriglyceridemia is of a magnitude comparable with or even larger than the contribution of intestinal lipoproteins is of interest. Similar findings have been described by Redgrave and Carlson (28) in humans. In the present study it was demonstrated that the postprandial increase in plasma of hepatic VLDL is not attributable to increased synthesis (which could have been the result of recycling of exogenous fatty acids taken up by the liver or the result of increased lipogenesis due to feeding). We suggest an alternative mechanism. It is well known that the rate of lipolysis by lipoprotein lipase of triacylglycerol-rich lipoproteins is related to size (29, 30). This is reflected in a very short half-life of chylomicrons as compared to hepatic VLDL. Therefore, we suggest that chylomicrons are the preferred substrate in the postprandial phase, resulting in an accumulation of hepatic VLDL during periods when both substrates are available. Subsequent studies on parameters of triacylglycerol catabolism revealed that rats fed the palm oil diet showed somewhat lower activities of lipoprotein lipase than sunflower seed oil-fed rats, in agreement with earlier observations (31, 32). However, differences in substrate type may be more important as sunflower seed oil lymph triacylglycerol is catabolized slightly faster than palm oil lymph triacylglycerol. Measurements were performed in recipient animals injected with mixtures of palm oil and sunflower seed oil lymph labeled in vivo with opposite C18:1 radioisotope precursors. The use of the same sets of C18:1 precursors for lymph labeling has obvious advantages as to known identical label stability, but introduces some risk for bias in results inasmuch as palm oil and sunflower seed oil contain different proportions of C18:1 (39% and 19%, respectively). However, differences in position of C18:1 incorporated into palm oil versus sunflower seed oil lymph triacylglycerol are unlikely to bias the data as presented in Table 3 as the hydrolysis of any fatty acid in a labeled triacylglycerol species is scored in our analysis method.

The mechanism by which small differences in catabolic rate between palm oil and sunflower seed oil lymph triacylglycerol could result in marked differences in postprandial plasma triacylglycerol concentrations needs special comment. Postprandial concentrations of triacylglycerol in plasma as observed in the present study are high (1–4 mM) compared to the K_m for triacylglycerol of muscle and adipose tissue lipoprotein lipase (0.07 mM and 0.7 mM, respectively) (33). This suggests that the lipolytic system in our rats is quite well saturated with substrate and indicates that a rise in substrate concentration will not result in a markedly increased flux. Under those conditions, small differences in rate of lipolysis of different substrates may lead to marked differences in dynamic concentrations of these substrates and this phenomenon may explain our data. The reason why sunflower seed oil chylomicrons are catabolized faster than palm oil chylomicrons may lie in the fatty acid composition of the triacylglycerol moiety. It has been shown recently (34) that the rate of lipolysis of chylomicrons in vivo varies with the degree of saturation of fatty acids present on the β position of glycerol in triacylglycerols. Hepatic VLDL may behave similarly, as marked effects in triacylglycerol fatty acid composition are expected between animals raised on palm oil or sunflower seed oil. In conclusion, the relative hypertriglyceridemia found in palm oil-fed rats is due to a less efficient triacylglycerol catabolism and not to increased rate of synthesis of plasma triacylglycerol.

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