Endogenous triglyceride-rich lipoproteins accumulate in rat plasma when competing with a chylomicron-like triglyceride emulsion for a common lipolytic pathway

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Abstract The rat liver secretes very low density lipoproteins (VLDL) containing either apoB-100 or apoB-48. After oral fat intake, chylomicrons containing apoB-48 and endogenously synthesized VLDL are mixed in the blood and the triglyceride clearance from these triglyceride-rich lipoprotein species compete for the same lipolytic pathway, i.e., lipoprotein lipase. A situation mimicking alimentary lipemia was induced by a short-term intravenous primed infusion of a chylomicron-like triglyceride emulsion to fed and fasted rats. The plasma concentration of apoB-100 and apoB-48 was monitored in triglyceride-rich lipoprotein subfractions after separation with density gradient ultracentrifugation by analytical SDS-PAGE. The net liver secretory output of VLDL was quantified by lipolytic blockade induced by Triton WR 1339. The chylomicron-like triglyceride emulsion induced a linear increase of large VLDL (S_f 60–400 subfraction containing both apoB-100 and apoB-48), almost to the same extent as that induced by Triton. The clearance of postprandial triglyceride-rich lipoproteins and both lipolysis and clearance of intravenously injected labeled rat chylomicrons was efficiently inhibited by the emulsion but not so complete as for fasting VLDL. The linearity of the VLDL increase and the very early response in the Intralipid-treated rats suggest that enhanced synthesis of VLDL is not a major cause for the accumulation. ■ Rather, the present data indicate that a high plasma concentration of a chylomicron-like triglyceride emulsion competes efficiently with liver-derived VLDL for the same lipolytic pathway, which leads to accumulation in plasma of endogenous VLDL in the postprandial state.—Karpe, F., and M. Hultin. Endogenous triglyceride-rich lipoproteins accumulate in rat plasma when competing with a chylomicron-like triglyceride emulsion for a common lipolytic pathway. *J. Lipid Res.* 1995. **36:** 1557–1566.

Supplementary key words apoB · apoC · apoE · alimentary lipemia · chylomicrons

The metabolism of triglyceride-rich lipoproteins leads to targeting of energy either for storage in adipose tissue or for immediate use in muscles. Remnants of triglyceride-rich lipoprotein particles acquire cholesterol that can be specifically diverted to tissues where it can be utilized for cell growth, hormone formation or bile acid production.

The liver secretes triglyceride-rich lipoproteins that have apoB as the major protein. ApoB exists in two molecular forms, apoB-100 and apoB-48, but there is one single gene encoding for both these apolipoproteins. The intestinal production of apoB-48 is the result of a tissue-specific apoB messenger RNA (mRNA) editing (1). In humans, apoB-100 is derived from the liver and secretion of chylomicrons containing apoB-48 is significant for the small intestine after absorption of dietary lipids. In rats, the hepatocytes have the apoB-100 mRNA editing capacity and therefore the hepatogenous secretion of triglyceride-rich lipoproteins from this species comprises both apoB-100 and apoB-48 (2). In addition, the rat intestine secretes triglyceride-rich apoB-48-containing lipoproteins after fat absorption.

Large triglyceride-rich lipoproteins, and in particular chylomicrons, are the favored substrates for lipoprotein lipase (LPL) (3). Essentially, all triglyceride-rich lipoproteins share the same lipolytic pathway, i.e., hydrolysis of the core triglyceride content by LPL. After a fat meal, chylomicrons and endogenous triglyceride-rich lipopro-

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teins (very low density lipoproteins, VLDL) are mixed in blood and thus compete for LPL. It has been shown that endogenous triglyceride-rich lipoproteins accumulate in human plasma after oral fat intake (4–6), but the mechanism behind this phenomenon is not obvious as both enhanced synthesis of VLDL and an impeded lipolysis have to be considered. In the rat, Robins et al. (7) have shown that the clearance of a short-term infusion of a triglyceride emulsion led to accumulation of endogenous plasma triglycerides, which indicated that the lipolytic system was saturated by the emulsion. In humans, Grundy and Mok (8) concluded that the triglyceride removal from plasma was not saturated after an intraduodenal infusion of a triglyceride emulsion (200 mg/h per kg body weight) whereas Brunzell and coworkers (9) showed that induction of hypersecretion of endogenous triglyceride-rich lipoproteins led to hypertriglyceridemia, taken as an indication of a common removal mechanism for triglyceride-rich lipoproteins, which is saturable.

In this study we address the issue of accumulation of triglyceride-rich lipoproteins after an oral fat load by using specific methods for identification of endogenous triglyceride-rich lipoprotein particles during experimental postprandial hypertriglyceridemia in the rat and comparing the results with complete inhibition of lipolysis in vivo. The experiments were short-term in order to rule out any confounding effect of enhanced VLDL secretion induced by the experimental procedures.

METHODS

Animals

Male Sprague-Dawley rats (Moellegaard Breeding Center, Ejby, Denmark) weighting 190–210 g were used in the experiments. The rats had been acclimatized in the animal house for at least 1 week before start of the experiment. They were kept on a 12-h light cycle (7 AM to 7 PM) and were allowed free access to standard pellet diet. When fasted rats were studied, the pellets were removed at 7 PM the day before the experiment. There were six rats in each group (Co, control; IL, Intralipid; Tri, Triton WR 1339). Experiments were performed on groups of fasted and fed rats separately. Rats were anesthetized by an intramuscular injection of Hypnorm (0.5 ml/kg bwt) and Stesolid Novum (2.5 mg diazepam/kg bwt) (Dumex, Copenhagen, Denmark). Hypnorm (Janssen Pharmaceutica, Bersee, Belgium) contains fentanyl citrate (0.315 mg/l) and fluanisone (10 mg/ml). All experimental procedures were approved by the local ethics committee (Umeå University).

Experimental procedures

All animal experiments were performed between 8 and 9:30 AM. Rats were anesthetized and a butterfly-cannula (Neofly®, Viggo-Spectramed, JMS, Japan) filled with saline was inserted into a lateral tail vein. An intravenous infusion of 1.0 ml of either Triton WR 1339 (Sigma) (100 mg/ml), Intralipid® (Pharmacia, Uppsala, Sweden) (100 mg/ml) + saline (0.75 ml + 0.25 ml) or saline alone (control) was given intravenously during 40 sec to the rat. The cannula was then flushed with 0.27 ml saline. A continuous infusion of Intralipid was started at a rate of 0.75 ml/h to rats that had received the bolus dose of Intralipid. The other groups received an infusion of saline at the same rate. Blood samples (approximately 450 µl) were taken from the left jugular vein before, 10, 20, and 30 min after the injection and transferred to tubes containing EDTA as anticoagulant. Rat plasma was immediately recovered by low speed centrifugation (1,750 g, 20 min, +1°C). Plasma triglyceride (TG) was determined with an enzymatic method (Boehringer Mannheim 877 577).

Isolation of triglyceride-rich lipoproteins

Lipoprotein fractions were prepared from plasma samples by density gradient ultracentrifugation, essentially as described by Redgrave and Carlson (10). A total volume of 0.15 ml of the plasma was put in the bottom of a 13.4 ml polyallomer ultracentrifuge tube (Ultra-Clear, Beckman Instruments, Palo Alto, CA) and gently mixed with 0.15 ml 1.21 kg/l NaCl. The diluted plasma was then mixed with 3.7 ml d = 1.10 kg/l and a density gradient consisting of 3.0 ml each of 1.065, 1.020 and 1.006 kg/l NaCl solutions was layered on top of the plasma. Ultracentrifugation was performed in a SW40 Ti swinging bucket rotor (Beckman) at 40,000 rpm and +15°C (Beckman L8-55 ultracentrifuge). Consecutive runs calculated to float $S_f > 400$ (32 min), S_f 60–400 (3 h 28 min) and S_f 20–60 (14–16 h) particles were made. After each centrifugation step, the top 0.5 ml of the gradient containing the respective lipoprotein subclass was aspirated, and 0.5 ml of density 1.006 kg/l salt solution was used to refill the tube before the next run. All salt solutions were adjusted to pH 7.4 and contained 0.02% NaN_3 and 0.01% Na_2EDTA. Densities were verified to the fourth decimal place (densitometer; Paar, Graz, Austria).

SDS-PAGE of isolated lipoprotein fractions

The apoB-48 and apoB-100 concentration in isolated fractions of triglyceride-rich lipoproteins was determined according to Karpe and Hamsten (11). In essence, isolated fractions of apoB-containing lipoproteins were delipidated in a methanol–diethylether solvent system. An aliquot of the lipoprotein fraction was delipi-

dated with 2.0 ml methanol and 2.0 ml of ice-cold diethylether, and centrifuged for 30 min at 4,000 g at +1°C. After removal of solvent, 2.0 ml of diethylether was added and the sample was centrifuged for another 20 min under the same conditions, whereafter the diethylether was removed, and the protein pellet was dissolved in 50 µl of 0.15 M sodium phosphate, 12.5% glycerol, 2% SDS, 5% mercaptoethanol, 0.001% bromphenolblue, pH 6.8, and denatured at +80°C for 10 min. Samples were run on 1.5 mm thick gradient (3–20%) polyacrylamide slab gels with a running buffer consisting of 25 mM Tris, 192 mM glycin, 0.2% SDS adjusted to pH 8.5 at 120 V for 2 h. Human apoB-100 isolated from low density lipoprotein (LDL) was used as standard. A standard curve consisting of six dilutions of apoB-100 was applied to each set of gels run together (0.1–2.0 µg). Gels were stained in 0.2% Coomassie G-250 (Serva, Heidelberg, Germany), 40% methanol, 10% acetic acid. Destaining was made in 12% methanol, 7% acetic acid. With this procedure, the chromogenicities of human apoB-48 and apoB-100 have been shown to be equal (11) and the rat apoB species was therefore assumed to behave likewise (12). The bands on the gel were scanned using a laser scanner (Ultroscan XL, Pharmacia-LKB, Sollentuna, Sweden) connected to a personal computer which was equipped with software providing automatic integration of areas under scanning curves (Gelscan XL, Pharmacia).

Influence of Intralipid on apoB-48 and B-100 measurements

To determine whether the presence of Intralipid in plasma influenced the fractionation and quantification of apoB-48 and apoB-100 in subfractions of triglyceride-rich lipoproteins, two aliquots of 0.8 ml of fasting plasma were incubated at 37°C for 30 min with either 50 µl 10% Intralipid or 46 µl distilled water and 6 µl glycerol (control sample). The volume of Intralipid added was calculated to correspond to a triglyceride elevation of approximately 7 mmol/l. Each incubate was then dispensed into three separate samples from which $S_f > 400$, S_f 60–400, and S_f 20–60 fractions were subsequently isolated for determination of the respective apoB-48 and B-100 concentrations. The contents of apoB-48 and apoB-100 in the S_f 60–400 subfraction were 1.1 ± 0.4 mg/l and 2.4 ± 0.2 mg/l for control samples and 1.3 ± 0.2 and 2.2 ± 0.3 mg/l for Intralipid-incubated samples, respectively. The corresponding concentrations in the S_f 20–60 fraction were 2.1 ± 0.7, 4.8 ± 0.7 and 2.0 ± 0.1, 5.0 ± 0.5 mg/l, respectively. Furthermore, there was no appearance of detectable amounts of apoB in the $S_f > 400$ fraction after incubation with Intralipid. The interpretation of these results is that the presence of Intralipid does not affect the isolation procedure of triglyceride-rich lipoproteins, nor do the lipoproteins acquire significant amounts of Intralipid-derived triglycerides, as that would lead to an increased flotation rate.

Chylomicron preparation

To prepare chylomicrons, the thoracic duct of an anesthetized rat was cannulated in the morning (13). During the operation another cannula was put into the stomach to allow for an intragastric infusion of 50 mg/ml glucose, 8.5 mg/ml NaCl, 0.5 mg/ml KCl at a rate of 2.5 ml/h. After the operation the rat was put in a restraining cage. During approximately 18 h, a volume of 80 ml lymph was collected (i.e., 4.4 ml/h). The next morning 100 µCi of [^{14}C]oleic acid and 100 µCi of [^{3}H]retinol (Amersham) were incorporated into 2 ml of Intralipid (100 mg/ml) by gentle mixing. The labeled triglyceride emulsion was given through the gastric tube. The flow of glucose/saline was continued until the end of chyle collection. The chyle was collected in sterile tubes containing EDTA (pH 7.4) and gentamycin (Garamycina®, Schering Corporation, Bloomfield, NJ) to a final concentration of 5 mM and 5 mg/ml, respectively. Twenty-four ml of labeled chyle was collected from 9 AM to 2 PM. The chyle was defibrinated and then centrifuged at 3000 rpm for 10 min at +4°C to remove white blood cells. The chyle was then layered under 9 mg/l NaCl, 50 µg/ml gentamycin and 5 mM EDTA and centrifuged in a Beckman SW 50 rotor at 30,000 rpm for 30 min at +4°C (14). The top layer was recovered and the chylomicrons were resuspended in the same buffer. More than 95% of the ^{14}C-label was contained in the TG fraction and 96% of the ^{3}H-label was retinyl esters (RE), as determined by separation of lipid extracts from the labeled chylomicrons on silica thin-layer chromatography plates with a system of heptane–diethylether–acetic acid 80:20:1.

Chylomicron clearance experiments

The rats were primed with a 10-min infusion of Intralipid/saline or treatment with Triton WR 1339 as described previously. Chylomicrons were injected into the right jugular vein. Blood samples were taken during 20 min and the blood was immediately extracted with 2 ml isopropanol–heptane–1 M H$_2$SO$_4$ 40:10:1 (v/v/v). At the end of the experiment tissues were removed, blotted dry, weighed, and then frozen at –20°C until homogenization in 30 vol. chloroform–methanol 2:1 (15). Blood samples were vortexed and 1.2 ml heptane and 1 ml of water were added (16). The tubes were vortexed and then centrifuged to separate water and organic solvent phases. One ml of the organic phase was added to 1 ml of alkaline ethanol (NaOH 50 mM in 50% ethanol) and the tubes were shaken and then centrifuged in order to

separate neutral lipids from free fatty acids (FFA). An aliquot of the top was taken for liquid scintillation and the bottom phase was washed twice with 3 ml heptane before scintillation. Four ml of scintillation liquid (Optiphase HiSafe III, LKB, Uppsala, Sweden) was added and the radioactivity was determined in an LKB-Wallac Rackbeta 1214 (LKB-Wallac, Uppsala, Sweden) using a built-in two-in-three dual isotope correction program.

Calculation and statistics

Conventional methods were used to calculate means and standard deviations (SD) and standard error of mean (SEM). In figures, SEM is indicated by bars. Computation of difference between groups was calculated with Student's t-test when two groups were involved and with analysis of variance with Scheffe's post-hoc test when three groups were compared. Student's paired t-test was used to identify differences within groups between time points.

Fractional catabolic rates (FCR) and half-lives were calculated by the SAAM II program using a fractional standard deviation for the data weighting of 0.05 and assuming a monoexponential decay of TG and core label (SAAM Institute, University of Washington, Seattle, WA).

RESULTS

Plasma triglycerides

The mean fasting plasma triglyceride level was just below 1 mmol/l in all groups irrespective of nutritional status (**Fig. 1**). Treatment with Triton WR 1339 induced a linear increase of plasma triglycerides and the rise was augmented by 50% in postprandial animals compared to fasting animals. The triglyceride level was unchanged when saline was infused, whereas a plateau at 8 mmol/l was achieved by the bolus and subsequent infusion of Intralipid in both fasting and postprandial animals (Fig. 1).

ApoB-100 and apoB-48 levels in triglyceride-rich lipoproteins

Base-line plasma from fasted animals contained both apoB-48 and apoB-100 in the S_f 60–400 and S_f 20–60 fractions. The levels of apoB in the $S_f > 400$ fraction was consistently lower than the detection limit (< 0.1 mg/l). ApoB-100 in the S_f 60–400 fraction increased linearly by approximately 300% during 30 min in response to both the Intralipid infusion and to the pretreatment with Triton WR 1339 in fasted rats (**Fig. 2**). The increase was already evident at 10 min ($P < 0.01$). The corresponding increase of apoB-48 was even greater, almost five times from the fasting level. Infusion of saline did not change the apoB levels in either subfraction. The accumulation of apoB-100 in the S_f 20–60 fraction after Triton treatment was less marked compared to the S_f 60–400 fraction. In the smaller VLDL subfraction, the initial effect of Intralipid was similar to the one of Triton, but at 20 min the increase of both apoB-48 and apoB-100 tended to level off.

Assuming that the accumulation in plasma of apoB-48 and apoB-100 due to Triton treatment reflected the synthetic rate and that all lipoprotein particles carried a single apoB molecule, it could be calculated that the rat liver secreted two apoB-48 lipoprotein particles for each apoB-100 particle and that two thirds of the secretion was confined to the S_f 60–400 fraction among the triglyceride-rich lipoproteins in the fasted rats.

Postprandial rats exhibited a different pattern (**Fig. 3**). The increase of apoB-48 in the S_f 60–400 fraction after pretreatment with Triton was enhanced and during the 30 min of observation the plasma concentration increased 5-fold in contrast to the accumulation of apoB-100, which was reduced to half of that seen in the fasting state. The apoB-48 concentrations in the $S_f > 400$ fraction did not mount to any significance (< 0.1 mg/l) in postprandial animals (results not shown). There was no accumulation of apoB-48 or of apoB-100 in the S_f 20–60 fraction, due to Triton treatment. Intralipid induced an increase of apoB-48 or apoB-100 in the S_f
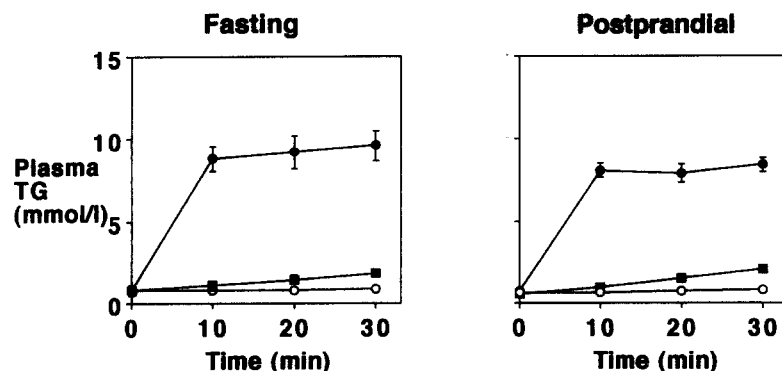

Fig. 1. Plasma triglycerides during the 30 min experiment in fasted and postprandial rats: (○) control; (■) Triton WR 1339 treatment; (●) Intralipid infusion. Values are mean ± SEM, n = 6.
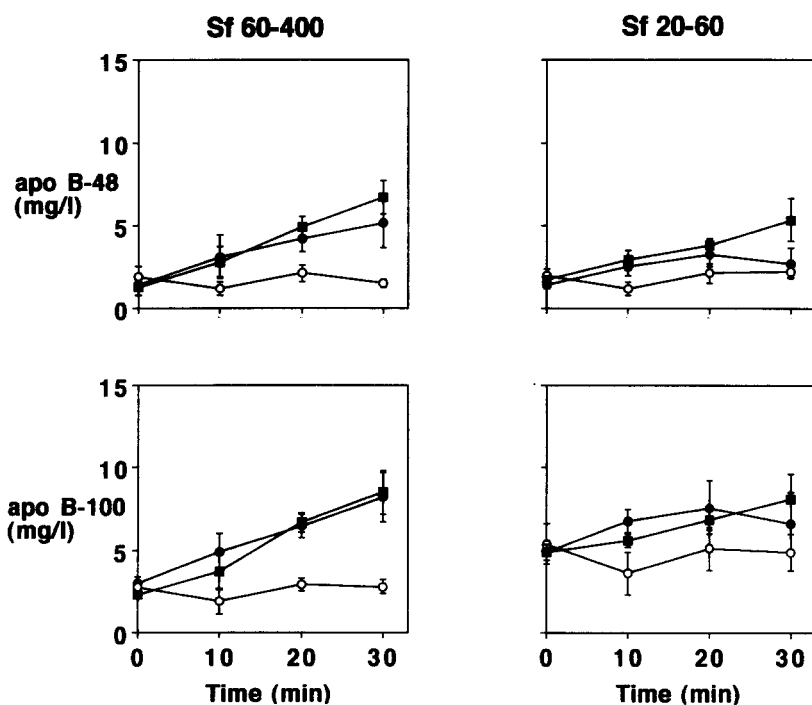
Sf 60-400

Sf 20-60

Fig. 2. Plasma concentration of apoB-48 and apoB-100 in S_f 60–400 and S_f 20–60 fractions in fasted rats after control (○), Triton WR 1339 (■), and Intralipid (●) treatments. Values are mean ± SEM, n = 6.

60–400 fraction similar to results with fasted rats but compared to Triton the increase was considerably smaller (≈50%). Furthermore, the concentration of both apoB-48 and apoB-100 increased in the S_f 20–60 fraction during the Intralipid infusion. The postprandial Triton-treated rats seemed to accumulate almost exclusively S_f

60–400 particles, i.e., the increase of both apoB-48 and apoB-100 in the S_f 60–400 fraction was 10-fold higher compared to the S_f 20–60 fraction.

It was noted that triglyceride-rich lipoproteins isolated from Triton-treated rats were almost devoid of apoE and contained very small amounts of apoC. A gel
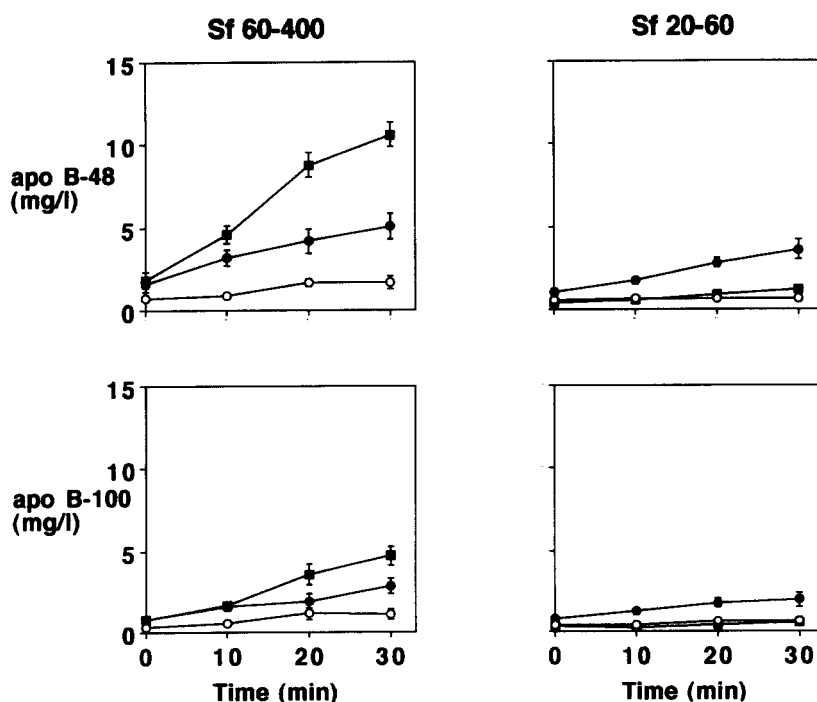
Sf 60-400

Sf 20-60

Fig. 3. Plasma concentration of apoB-48 and apoB-100 in S_f 60–400 and S_f 20–60 fractions in postprandial rats after control (○), Triton WR 1339 (■), and Intralipid (●) treatments. Values are mean ± SEM, n = 6.

showing representative lipoprotein samples from Control, Intralipid- and Triton-treated rats is shown in **Fig. 4.**

Chylomicron clearance

The dual label in the chylomicrons allowed us to investigate both the lipolysis step, caused by the action of LPL, and the tissue uptake of lipoprotein remnants. In control rats RE and TG disappeared with FCRs of 0.042 ± 0.005 min^{-1} and 0.111 ± 0.011 min^{-1}, respectively. Some of the TG label reappeared in the FFA fraction reaching a peak of 2.5% after 3–5 min. This rapid recirculation of TG label in the FFA fraction reflects the action of LPL on the labeled chylomicrons (14, 17). When rats were treated with Triton WR 1339, both lipolysis and particle uptake were virtually abolished (FCR-RE 0.009 ± 0.007 min^{-1} and FCR-TG 0.011 ± 0.003 min^{-1}) (**Fig. 5**). The decreased lipolysis was also reflected by the low amount of label found in the FFA fraction. Consistently, 0.5–0.7% of the TG label was found in the FFA fraction, probably reflecting the presence of FFA on the surface of chylomicron particles. In rats receiving the primed infusion of Intralipid, the disappearance of RE (FCR 0.018 ± 0.004 min^{-1}) and TG was slow (FCR 0.028 ± 0.002 min^{-1}). The disappearance of RE and TG in Intralipid-treated rats was significantly slower compared to saline, but not to Triton. The amount of label recirculating in the FFA fraction in the Intralipid-treated rats was lower to begin with, but increased with time. Three min after the injection, 0.8% of the TG label was found in the FFA fraction, while 10 min after injection 1.3% of the TG label was recovered as FFA. Accordingly, the patterns of TG and RE disappearance were fairly similar when Intralipid- and Triton-treated rats were compared. The appearance of FFA was, however, more prominent in the Intralipid-treated rats.

The differences in blood clearance of chylomicrons was also reflected by the differences in the uptake of chylomicrons by the liver. Despite the fact that almost no lipolysis occurred in the Triton-treated rats, as much as 8.0% of the injected chylomicrons was recovered in the liver 20 min after injection (**Table 1**). Compared to control rats, in rats receiving Intralipid or Triton treatments the liver uptake was approximately one fourth of the chylomicron particle label (38%, 13.4 and 8.0% of RE, respectively). The differences between the TG label recovered in the liver were less marked; 16.8%, 9.7%, and 7.3% (Control, Intralipid, and Triton, respectively). The quotient of TG over RE indicates the balance between TG uptake and particle uptake (RE). In epididymal fat pads, the saline-treated rats had a ratio of 2.2, indicating a selective uptake of TG, while the corresponding ratio for the liver was 0.44, which indicates an uptake of remnant particles. The corresponding ratios for Intralipid- and Triton-treated rats were 1.36, 1.48 and 0.74, 0.91, respectively, which indicate that the specific distribution of TG to adipose tissue and remnant uptake in the liver was partially abolished. The total deposition of chylomicron particles in the liver was smaller in rats treated with Intralipid or Triton (lower RE label), but the ratio was higher. This indicates that the few chylomicron remnant particles taken up by the liver during treatment were considerably more triglyceride-rich.

DISCUSSION

The present work deals with the consequences of competition for lipolysis in plasma between triglyceride-rich lipoproteins of various origin. In essence, a high plasma concentration of a chylomicron-like triglyceride emulsion leads to accumulation of endogenously synthesized liver-derived VLDL in the rat plasma. The mechanism of action is not obvious, as both enhanced synthesis of VLDL due to the increased substrate delivery to the liver as well as a reduced catabolism of this lipoprotein species should be considered. The current hypothesis is that the triglyceride content of chylomicrons and VLDL is hydrolyzed prior to the uptake of
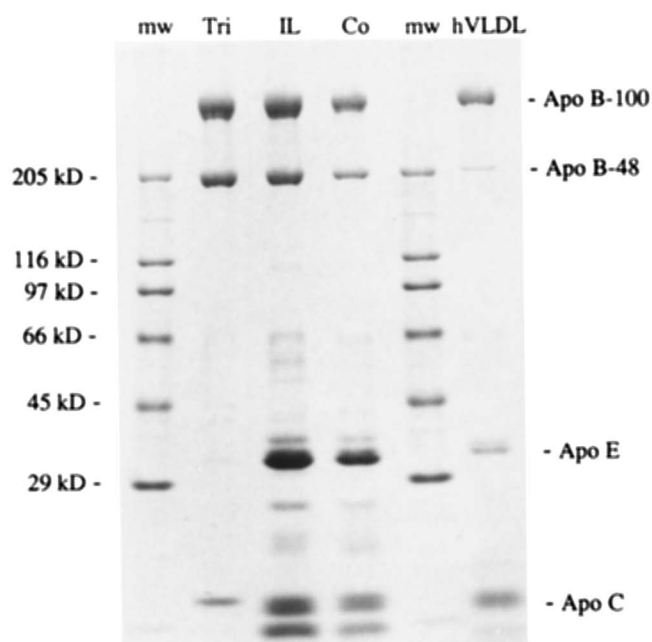

Fig. 4. An SDS-PAGe gel showing apoB-100, B-48, E and Cs from isolated triglyceride-rich lipoprotein samples. Lanes 1–5 from left to right: mw, high molecular weight standard from Sigma; Tri, S$_f$ 60–400 fraction 30 min after Triton WR 1339 treatment; IL, S$_f$ 60–400 fraction 30 min after initiation of Intralipid infusion; Co, S$_f$ 60–400 fraction after 30 min treatment with saline infusion; hVLDL, a non-fasting human S$_f$ 60–400 fraction.
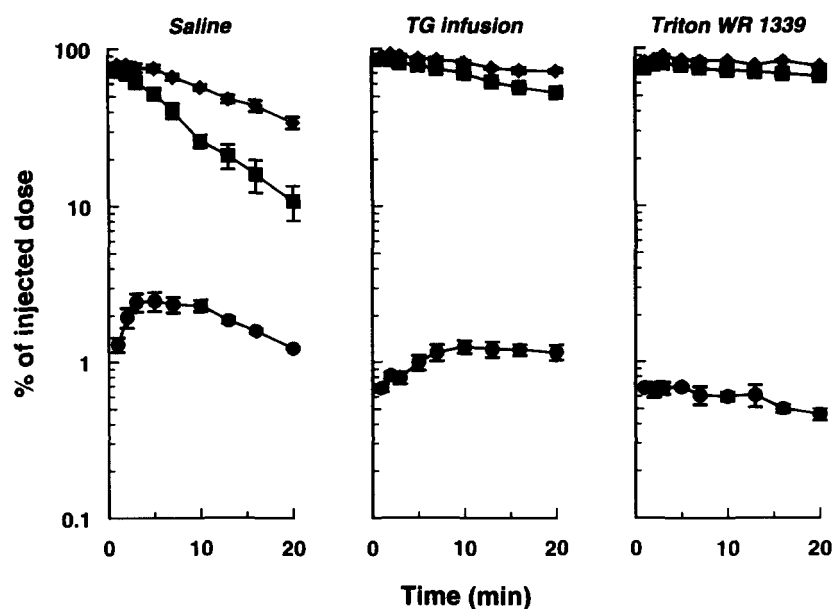
Fig. 5. Effect of infusion of a chylomicron-like triglyceride emulsion (Intralipid), treatment with Triton WR 1339 or saline on turnover of doubly labeled chylomicrons. Chylomicrons were labeled with triglycerides (TG) or retinyl esters (RE). Chylomicrons (4 mg TG) was given to fed rats 10 min after the start of the infusion of the triglyceride emulsion or administration of Triton. The clearance of [^{14}C]TG (■) and [^{3}H]RE (◆) and the appearance of ^{14}C-labeled free fatty acids (●) were followed. Values are mean ± SEM, n = 6.

remnant lipoproteins by specific receptors in the liver. Accordingly, the presence in plasma of large amounts of chylomicron-like triglyceride emulsion particles may lead to reduced catabolism of VLDL due either to competition for lipolysis or to competition for uptake at the receptor level.

TABLE 1. Tissue distribution of labeled chylomicrons in control animals and after Intralipid and Triton WR 1339 treatment

Tissue	Treatment		
	Control	Intralipid	Triton WR 1339
[^{3}H]retinyl ester label			
Liver, per organ	38.3 ± 2.1	13.4 ± 1.4[a]	8.0 ± 0.7[a]
Lung, per organ	0.9 ± 0.1	1.1 ± 0.1	1.5 ± 0.3[a]
Heart, per organ	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.0
Spleen, per organ	1.7 ± 0.1	2.0 ± 0.1	2.4 ± 0.2[a]
Kidney, per organ	0.5 ± 0.1	0.8 ± 0.0[a]	0.9 ± 0.1[a]
Diaphragm, per g tissue	1.4 ± 0.3	1.5 ± 0.2	1.4 ± 0.1
Epididymal fat pad, per g tissue	1.1 ± 0.2	1.0 ± 0.1	1.2 ± 0.2
[^{14}C]triglyceride label			
Liver, per organ	16.8 ± 0.6	9.7 ± 0.7[a]	7.3 ± 0.5[a,b]
Lung, per organ	0.6 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
Heart, per organ	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.1
Spleen, per organ	1.1 ± 0.1	1.3 ± 0.1	1.6 ± 0.2
Kidney, per organ	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0
Diaphragm, per g tissue	0.9 ± 0.1	0.7 ± 0.1	0.6 ± 0.0
Epididymal fat pad, per g tissue	2.2 ± 0.2	1.4 ± 0.3	1.7 ± 0.4
^{14}C/^{3}H label			
Liver, per organ	0.44 ± 0.02	0.74 ± 0.03[a]	0.91 ± 0.03[a,b]
Lung, per organ	0.66 ± 0.05	0.69 ± 0.05	0.60 ± 0.07
Heart, per organ	0.57 ± 0.08	0.42 ± 0.03	0.46 ± 0.08
Spleen, per organ	0.66 ± 0.02	0.63 ± 0.06	0.65 ± 0.05
Kidney, per organ	0.71 ± 0.08	0.37 ± 0.06[a]	0.38 ± 0.04[a]
Diaphragm, per g tissue	0.67 ± 0.12	0.53 ± 0.06	0.46 ± 0.04
Epididymal fat pad, per g tissue	2.16 ± 0.21	1.35 ± 0.24	1.48 ± 0.32

Tissue samples were taken from the rats 20 min after injection of labeled chylomicrons. The tissue samples were rinsed in saline, blotted dry, weighed, and frozen until analysis. The amount of radioactivity was determined after homogenization and extraction in 30 volumes of chloroform–methanol 2:1. The ratio of ^{14}C to ^{3}H in each organ was calculated as an indication of particle uptake versus lipolysis in each organ. Statistical differences were tested for by analysis of variance followed by the post-hoc test Scheffe. Values are mean ± SEM.
[a]$P < 0.05$ versus saline.
[b]$P < 0.05$ versus Intralipid.

The effect of the non-ionic detergent Triton WR 1339 on the accumulation of endogenous triglyceride-rich lipoproteins, which is likely to depend on a disruption of the interface between LPL and lipoproteins and thereby inhibiting lipolysis (18), was compared with intravenous administration of a high dose of a chylomicron-like triglyceride emulsion. In fasting animals apoB-100 in large VLDL (presumably newly synthesized) accumulated linearly during 30 min in an indistinguishable manner comparing the Triton and Intralipid treatments. The corresponding accumulation of apoB-48 due to Intralipid treatment was slightly lower, but statistically not significantly different from the Triton-treated rats. The linearity and the very early response in the Intralipid-treated rats strongly suggest that enhanced synthesis is not a major cause for the accumulation. An enhanced liver-derived VLDL secretion is not compatible with the fact that the secretory pathway of VLDL from rat hepatocytes takes 20–40 min (19). It is therefore likely that the large amount of chylomicron-like emulsion particles becomes the favored substrate for LPL and thereby saturates the lipolytic system. The accumulation of apoB-48 and apoB-100 induced by Intralipid was less marked among the smaller VLDL species, but Triton treatment elicited a linear increase of apoB-48 and apoB-100 that was about half of that seen in the large VLDL fraction. Initially, Intralipid caused a similar accumulation, but the curve leveled off at 20 min. One explanation for this finding could be that a small-sized VLDL particle is also a good substrate for hepatic lipase, for which the chylomicron-like emulsion particles presumably are poor competitors (3).

With the assumption that a lipoprotein particle contains only one apoB molecule, it could be calculated that the secretory output of triglyceride-rich lipoproteins from the fasting rat liver in vivo constitutes of two apoB-48 particles for each B-100 particle and that two thirds are large and triglyceride-rich particles (S_f 60–400). The accumulation of apoB-100 in the S_f 60–400 fraction was attenuated in postprandial animals. This might be attributed to effects of insulin as the rat pellet diet is fairly carbohydrate-rich. Insulin is known to attenuate the secretion of triglyceride-rich lipoproteins from hepatocytes in the acute phase (20, 21).

Postprandial rats exhibited a different pattern. As there is also an input of apoB-48 particles from the intestine, the origin of the accumulating apoB-48 particles found during Triton treatment cannot be known. As expected, the postprandial accumulation of apoB-48 in S_f 60–400 after Triton treatment was considerably enhanced. The triglyceride emulsion did not, however, evoke a similar response, instead the accumulation was half of that in the Triton group. Of note, there was a small accumulation of apoB-48 in the S_f 20–60 fraction when Intralipid was given. As addition of the accumulation of apoB-48 in the S_f 20–60 and S_f 60–400 subfractions in the Intralipid-treated groups almost mounted to the accumulation seen in the S_f 60–400 fraction in the Triton-treated animals, the smaller lipoprotein particles were presumably not derived from de novo synthesis but converted from the larger species despite the presence of the chylomicron-like triglyceride emulsion. This fits well with the notion that chylomicrons are favored in the lipolytic system compared with VLDL. The question also arises as to whether this favor only depends on the size of chylomicrons or whether the specific apolipoprotein composition of triglyceride-rich lipoprotein from the intestine leads to an enhanced interaction with LPL.

In the present study, the metabolism of rat chylomicrons was investigated under the same conditions as described for endogenous triglyceride-rich lipoproteins. Triton did indeed block lipolysis, as it was shown that there was almost no generation of chylomicron-derived FFA as well as persisting levels of TG and RE after injection of labeled chylomicrons. The in vivo mixing in plasma of true chylomicrons with an excess of chylomicron-like triglyceride emulsion particles led to a grossly impaired lipolysis of chylomicrons. Furthermore, the use of RE labeling enabled us to study the particle uptake in tissues. The liver uptake of chylomicrons was partially blocked by Triton. Several explanations seem plausible. First, the total lack of lipolysis may cause a steric hindrance for the very large particles to pass the sinusoidal fenestra in the liver. Second, chylomicrons from Triton-treated rats seemed to lose apoE and C, i.e., the putative ligand to the chylomicron remnant receptor and the cofactor for LPL. In Intralipid-treated rats the liver uptake of chylomicron remnants was impaired almost to the extent of Triton treatment. It is therefore tempting to speculate that the impaired lipolysis is one major explanation and that loss of apoE and C to the emulsion particles could be another. It is well known that apoC and E transfer between particles according to the need (22) and that Intralipid acts similar to chylomicrons in this respect (23). Furthermore, one of us has previously postulated that the defective metabolism of postprandial triglyceride-rich lipoproteins among hypertriglyceridemic patients with coronary heart disease is partly due to a relative postprandial deficiency of apoC and E in the pool of triglyceride-rich lipoproteins (5).

An estimate of the TG transport rate was made by multiplying total plasma TG mass with the FCR of control, Triton- and Intralipid-treated rats. It must then be assumed that Intralipid triglycerides are cleared at the same rate as chylomicron triglycerides. The transport rates were 0.68 mg/min, 0.07 mg/min, and 1.36 mg/min, respectively. Intralipid was infused at a rate of

1.25 mg/min while the plasma TG concentration was stable, which indicate the fairness of the calculated TG transport rates. Despite the doubled TG transport rate comparing control and IL rats, VLDL apoB, C, and E masses increased. This may reflect the fact that IL particles are lipolyzed preferentially, leaving endogenous VLDL unlipolyzed and that the accumulating pool of VLDL carried apoC and E recruited from HDL. A further explanation for the increased TG transport rate in the IL-treated rats is that partially lipolyzed IL particles are removed, which may then add to the total TG removal rate. A corresponding situation is found for the metabolism of human chylomicrons as they seem to be removed at a stage when they still carry a large amount of TG (24).

Robins et al. (7) have studied the competition between endogenous triglyceride-rich lipoproteins and a chylomicron-like triglyceride emulsion. They claimed that the accumulation of endogenous triglycerides was due to competition for a common lipolytic pathway with the chylomicron-like emulsion particles, which were labeled with specific fatty acids to trace the lipolytic products in plasma. That represents an indirect evidence, and actual quantification of triglyceride-rich lipoprotein particles was not made in those experiments. A similar conclusion was reached in humans in a classical study performed by Brunzell and coworkers (9). They found evidence for a common, saturable, triglyceride removal mechanism for chylomicrons and VLDL in humans by increasing the VLDL production rate with a carbohydrate-rich diet. With this treatment hypertriglyceridemic subjects were found accumulate large VLDL. One assumption was that the removal capacity of VLDL TG was constant over weeks; whether this was correct or not could be discussed. In contrast, Grundy and Mok (8) found no signs of saturability in the triglyceride removal in subjects undergoing intraduodenal infusion of a triglyceride emulsion. In that study it was assumed that a steady state condition was attained after 5 h infusion. With todays' knowledge this assumption is questionable as it is known that a subpopulation of the pool of endogenous triglyceride-rich lipoproteins is expanding in the postprandial setting (5, 6). Furthermore, the mechanism of removal may change in the postprandial state. Indeed, Grundy and Mok (8) found evidence for differences in the clearance process of VLDL particles in contrast to remnants of chylomicrons, in agreement with what one of us has recently shown (24).

The present results help to clarify this issue as it was shown that lipolysis of endogenous triglyceride-rich lipoproteins was severely delayed by the presence of a chylomicron-like triglyceride emulsion, and that a major explanation for the postprandial increase of their plasma concentration could be attributed to a failure to compete for a common lipolytic pathway. ■

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