Chylomicron metabolism in an animal model for hyperlipoproteinemia type I

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Abstract Mink homozygous for the mutation Pro214Leu in lipoprotein lipase (LPL) had only traces of LPL activity but amounts of LPL protein in their tissues similar to those of normal mink. In normal mink, lymph chylomicrons from rats given [^{3}H]retinol (incorporated into retinyl esters, providing a core label) and [^{14}C]oleic acid (incorporated mainly in triglycerides (TG)) were rapidly cleared from the circulation. In the homozygous mink, clearance was much retarded. The ratio of TG to core label in plasma did not decrease and much less [^{14}C]oleic acid appeared in plasma. Still, half of the labeled material disappeared from the circulating blood within 30–40 min and the calculated total turnover of TG in the hypertriglyceridemic mink was almost as large as in normal mink. The core label was distributed to the same tissues in hypertriglyceridemic mink as in normal mink. Half to two-thirds of the cleared core label was in the liver. The large difference was that in the hypertriglyceridemic mink, TG label (about 40% of the total amount removed) followed the core label to the liver and there was no preferential uptake of TG over core label in adipose or muscle tissue. In normal mink, only small amounts of TG label (<10%) appeared in the liver, while most was in adipose and muscle tissues. Apolipoprotein B-48 dominated in the accumulated TG-rich lipoproteins in blood of hypertriglyceridemic mink, even in fasted animals.—Savonen, R., K. Nordstoga, B. Christophersen, A. Lindberg, Y. Shen, M. Hultin, T. Olivercrona, and G. Olivecrona. **Chylomicron metabolism in an animal model for hyperlipoproteinemia type I. *J. Lipid Res.* 1999.** 40: **1336–1346.**

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Chylomicrons are triglyceride (TG)-rich lipoproteins formed by intestinal mucosal cells during absorption of dietary fat (1, 2). After secretion into extracellular fluid and mesenteric lymph, the chylomicrons are transported through *ductus thoracicus* to the blood circulation. There they are depleted of TG by the action of lipoprotein lipase (LPL, E.C 3.1.1.34) resulting in smaller, denser chylomicron remnants (3, 4). The released fatty acids are taken up by the adjacent tissue or are returned to plasma to join the albumin-bound pool of free fatty acids (FFA) (5). LPL is synthesized mainly in adipose and muscle tissue and is located bound to heparan sulfate proteoglycans on the luminal side of the vessel walls (6, 7). The synthesis and post-translational formation of active LPL is regulated by the nutritional status so that the enzyme is more active in adipose tissue in the fed state than in the fasted state (8–10). To act on lipoproteins, LPL requires an activator, apolipoprotein C-II, which is normally present in sufficient amounts on the substrate lipoproteins (11).

Deficiency of LPL, or of apolipoprotein C-II, leads to hyperlipoproteinemia type I characterized by grossly elevated fasting TG levels in the chylomicron/chylomicron remnant fraction. In humans, LPL deficiency is a rare autosomal recessive disease with a frequency of about $1/10^6$ in the general population (12, 13). The clinical picture in these patients, with TG levels over 1500 mg/dl (17 mmol/l), involves recurrent abdominal pain, acute pancreatitis that may be fatal, eruptive xanthomata, and lipemia retinalis. Hepatomegaly is common and splenomegaly may also occur. The symptoms are ameliorated by a low fat diet. Clearance of chylomicrons in patients with hyperlipoproteinemia type 1 was previously found to be greatly retarded when studied after infusion of radiolabeled human chylomicrons (14) or after administration of retinyl palmitate per os (15).

Attempts to generate a mouse model deficient in LPL by gene targeting (16, 17) have not been successful as the mice die shortly after they start to suckle. Mice carrying the *cld*/*cld* mutation, which causes lack of both LPL and

Abbreviations: apoB, apolipoprotein B; CETP, cholesteryl ester transfer protein; E%, energy percent; FFA, free fatty acids; FCR, fractional catabolic rate; LPL, lipoprotein lipase; TG, triglyceride.

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hepatic lipase activity, also die shortly after birth (18, 19). Expression of LPL in a single tissue, e.g., in skeletal muscle or in the heart, is, however, enough to rescue the LPL knock-out mice (20).

A strain of domestic cats carrying a mutation in the LPL gene leading to a catalytically inactive enzyme protein has been described (21–25). The clinical picture in the LPL-deficient cats differs from that in humans as the cats do not develop pancreatitis but suffer from neurological symptoms due to affection of dorsal root ganglions (23).

Previously we reported on a strain of hypertriglyceridemic mink with deficiency of active LPL (26). Affected animals have lesions in abdominal organs that seem to be secondary to massive accumulation of lipids. Some animals develop pancreatitis. A single point mutation was found in the LPL gene of affected animals resulting in a Pro214Leu substitution (27). Homozygotes have no LPL activity in pre- or post-heparin plasma. LPL protein is present in plasma, but in an inactive form (26). Activity of hepatic lipase is normal in post-heparin plasma. Heterozygotes have normal plasma lipid levels though they have decreased levels of LPL activity in some tissues including post-heparin plasma (27). Mink have very low levels of cholesteryl ester transfer protein (CETP) activity in plasma compared to humans (27).

In the present study we measured lipase activity and LPL mass in tissues from affected (homozygote) mink. To investigate the effect of LPL deficiency on chylomicron metabolism, we injected radiolabeled rat lymph chylomicrons intravenously into normal and hypertriglyceridemic mink. The objective was to determine in what tissues, and if possible also how, animals with functional LPL deficiency metabolize chylomicrons. Another question was whether hepatic lipase could compensate for the lack of LPL.

METHODS

Materials

[11,12, ^{3}H(N)]retinol and [1-^{14}C]oleic acid were from Amersham, U.K. [^{51}Cr] as chromium chloride was from DuPont-NEN, USA. Bovine serum albumin (fraction V) was from Sigma, St. Louis, MO; leupeptin and pepstatin were from Peptide Institute Inc., Osaka, Japan; aprotinin (Trasylol®) was from Bayer, Leverkusen, Germany; protease inhibitor cocktail tablets Complete™, Mini, were from Boehringer Mannheim, Germany; heparin was from Lövens, Malmö, Sweden; and Triton X-100 was from Riedel de Haen, Seelze, Germany. Heparin-Sepharose was made according to the method of Iverius (28). All chemicals were of analytical grade.

Animals

Standard mink *(Mustela vison)* were kept in outdoor cages in sheds until 1 week before the experiments. Mink mate only once a year and give birth to about four kits. Therefore, the number of kits available for our experiments was quite limited. After weaning, at around 8 weeks of age, the kits were separated from their mother and given only standard mink diet. Adult, female mink weigh up to 1 kg whereas males can weigh up to about 2 kg. Depending on the raw materials available for the producers, the diet contained 28–39 E% protein, 32–55 E% fat, and 18–29 E% carbohydrates. Adult mink consume about 200 g (wet weight) food per day.

Earlier (26) and during the first year of the present studies, homozygote mink were identified by the appearance of turbid plasma. Among the kits born in 1995 (around 50), two homozygote mink were identified in this manner at about 8 weeks of age. These two mink, together with four non-related normal mink (group A in Table 3) were put on a fat-reduced diet (24 E% fat) for about 10 days, to reduce plasma TG. One of the affected mink failed to thrive on the diet and started self-mutilating her tail after a few days. To prevent infection she was treated with Tribessen® vet. (Trimetoprim + sulfonamid, Pitman-Moore, Ireland) per os for 3 days. Then, at 14 weeks of age, the animals were fasted overnight and used in the experiment shown in Figs. 2–4 and in Tables 3 and 5.

In 1996 four homozygote mink were found among 79 littermates as indicated by the appearance of turbid plasma. Genotyping (27) confirmed the diagnosis and also revealed a fifth animal homozygous for the mutation. Repeated blood sampling showed that this mink was also hypertriglyceridemic. This mink was not used in the experiments presented here, but was kept for breeding. Another of the homozygote mink died at 12 weeks of age. Autopsy indicated that pancreatitis-peritonitis was the cause of death. Therefore, experiments (Table 4) were carried out on three LPL-defective and eight normal (confirmed by genotyping) mink (group B in Table 3). These mink were kept on a standard mink diet with 55 E% fat until the experiment was performed. In addition, studies were made on an unrelated group of normal mink (Fig. 1, Tables 1 and 2). These mink were not related to the hypertriglyceridemic mink and were kept at a separate location on the farm. No hypertriglyceridemic mink has ever been found among these animals. These animals were not genotyped (used for experiments before 1996). For further characteristics of normal and LPL-deficient mink, see Christophersen et al. (26). All deficient mink used for the experiments had enlarged, pale yellowish livers but no granulomas. The liver weights for homozygotes in group A were 6.39 ± 3.86% of total body weight compared to 2.02 ± 0.36% for the controls. For group B the corresponding numbers were 4.82 ± 1.25% compared to 2.64 ± 0.23%, indicating that the livers had accumulated considerable amounts of lipids.

The mink experiments were carried out at the Norwegian College of Veterinary Medicine, Oslo, Norway. The animals were anesthetized by intramuscular injection of Ketalar® (ketamin, Park Davis, Barcelona, Spain) 10 mg/kg and Domitor® vet. (medetomodin + metylparahydroxybensoate, Farmos, Åbo, Finland) 0.2 mg/kg. Chylomicrons were injected in an exposed jugular vein. Blood samples were taken from the other jugular vein. A final, larger blood sample was taken, in EDTA-containing tubes, from the heart. The animals were then killed by an overdose of Domitor and hearts and other tissues were removed and weighed. A small aliquot was immediately frozen on dry ice or in liquid nitrogen. These samples were shipped to Umeå for analysis of lipase activity and LPL mass as described below. The rest of the organ, or a sample of it, was taken for extraction of radioactivity either directly in Oslo, or frozen and shipped to Umeå. Samples were also taken for genotyping (27) and histopathological examination.

The mink experiments were approved by the laboratory animal science specialist under the surveillance of the Norwegian Experimental Animal Board and registered by the board. The experiments were conducted in accordance with the laws and regulations controlling experiments in live animals in Norway.

Rat chylomicrons

Male nonfasting Sprague-Dawley rats (Møllegard Breeding Centre, Skensved, Denmark) weighing 220–280 g were used for

preparation of chylomicrons as described by Hultin et al. (29). Briefly, the rats were anesthetized and plastic tubes were inserted into the thoracic duct and into the stomach. After the rats had recovered, 150 μCi [^{3}H]retinol and/or 150 μCi [^{14}C]oleic acid in 4–5 ml 10% Intralipid® (Pharmacia-Upjohn, Stockholm, Sweden) was infused intragastrically. Lymph was collected during 5–7 h. Chylomicrons were then isolated by ultracentrifugation through 0.154 m NaCl, 0.05% EDTA, and 0.01% Gentamicin® (Schering Corporation USA, Kenilworth, NJ). More than 92% of the [^{3}H]retinol in the chylomicrons is present as retinyl esters and provides a label for the core material (29). [^{14}C]oleic acid is incorporated primarily in the TG of the chylomicrons (more than 93% (29)). These procedures were approved by the animal ethics committee of Northern Sweden. The chemical amount of TG was determined by an enzymatic kit from Boehringer Mannheim, Germany. The doubly labeled chylomicrons contained about 400 dpm [^{3}H]retinol and about 600 dpm [^{14}C]oleic acid per nmol TG. The amount of chylomicrons injected into the mink corresponded to 20 mg TG per kg body weight.

Plasma lipid analyses

Triglycerides and cholesterol were analyzed by enzymatic methods (Boehringer Mannheim) at the Institute of Clinical Biochemistry at Rikshospitalet in Oslo.

Lipase measurements

The substrate used for assay of LPL activity was a phospholipid-stabilized TG emulsion prepared by Pharmacia & Upjohn, Stockholm, Sweden. It had the same composition as the commercial 10% Intralipid® but contained trace amounts of [^{3}H]oleic acid-labeled triolein. The conditions were otherwise as described previously (pH 8.5, 25°C, rat serum as source of apolipoprotein C-II) (26). The frozen tissue pieces were thawed and homogenized with a Polytron homogenizer (PT-MR 3000, Kinematica, Littau, Switzerland) in a volume of nine times their weight of ice-cold buffer (0.025 m NH_3, 5 mm EDTA, adjusted to pH 8.2 with dilute HCl and containing per ml: 1 mg bovine serum albumin, 10 μg leupeptin, 1 μg pepstatin, 25 IU aprotinin, 5 IU heparin, 10 mg Triton X-100, and 1 mg sodium dodecyl sulfate). The homogenate was centrifuged for 10 min at 3,000 rpm in a Heraeus Minifuge-T centrifuge (Heraeus Christ, Osterode, Germany). LPL activity was measured in the subphase collected between the layer of floating fat and the pellet of tissue debris. Hepatic lipase displays partial activity in this assay. None of our antisera against hepatic lipase from other species reacted well with hepatic lipase from mink. Therefore we could not use immunoinhibition to suppress hepatic lipase (26). One milliunit of lipase activity corresponds to 1 nmol/fatty acids released per min.

LPL mass was measured by an enzyme-linked immunoassay as previously described (26, 30).

Chromatography of tissue homogenates on heparin-Sepharose

Frozen tissues (1.3–2.9 g) were homogenized in buffer containing detergents as described above, but without heparin and with the commercial protease inhibitor cocktail, Complete™, Mini (1 tablet/50 ml) instead of those described above. After centrifugation the homogenates were applied to columns containing 2 ml heparin-Sepharose and eluted by linear gradients (40 + 40 ml, 0–1.6 m NaCl) as previously described (26). Lipase activity and LPL mass were analyzed in the fractions. For fractions from tissues of hypertriglyceridemic mink, large aliquots (10 μl) and long incubation times (2 h) were used to enable detection of LPL activity.

Lipid extractions

Blood samples (200–300 μl) were immediately transferred to tubes containing 1.2 ml isopropanol–heptane–1 m H_2SO_4 40:10.1, and further treated and separated into fatty acid esters and unesterified fatty acids as described by Hultin, Savonen, and Olivercrona (5). Tissues were homogenized using a Polytron (Kinematica, Basel, Switzerland) and extracted in 30 times the tissue weight of chloroform–methanol 2:1 (v/v). The samples were further treated to recover lipid-soluble label as previously described (5). Aliquots of the final lipid extracts were dried and scintillation liquid (OptiPhase Hisafe III, Pharmacia, Uppsala, Sweden) was added. Samples were then simultaneously counted for ^{3}H and ^{14}C radioactivity in an LKB-Pharmacia 1214 β-counter.

Radioactivity in tissues was corrected for the contribution from blood remaining in the respective organ. For this, the amount of blood in tissues of a separate group of normal mink, treated as in the other experiments, was determined using ^{51}Cr-labeled red blood cells (from mink) as previously described (5). The blood volume was found to be 7.8% of the weight of the animal.

Separation of lipoproteins by centrifugation and delipidation for SDS-PAGE

Lipoproteins were isolated from fresh EDTA-plasma by ultracentrifugation. Large, TG-rich chylomicrons were recovered from plasma from two homozygote animals (in 1998) after centrifugation for 30 min at 20,000 rpm, followed by a wash at the same density. The fat cake at the top after the wash was collected as the chylomicron fraction. The less rapidly floating lipoproteins in the subphase were recovered as light VLDL. Remaining plasma was centrifuged for 18 h at 40,000 rpm (d 1.006 g/ml) for flotation of VLDL. They were washed once under the same conditions. All three fractions were delipidated and prepared for SDS-PAGE according to Karpe and Hamsten (31) and gels containing 7.5% polyacrylamide were run using prestained SDS-PAGE molecular weight standard, high range, from Bio-Rad. For Western blot, proteins were transferred to an Immobilon filter as described (32). The filter was blocked with 5% (w/v) bovine serum albumin and 3% (w/v) gelatin in 20 mm Tris, 0.5 m NaCl, 0.005% Tween-20 at pH 7.5. ApoB was detected by a rabbit polyclonal antiserum raised against human apoB (DAKO A/S, Denmark, diluted 1/500) followed by a secondary alkaline phosphatase-conjugated goat anti-rabbit IgG (diluted 1/3000).

Kinetic analysis

Data for radioactivity in blood after injection of labeled chylomicrons to normal mink was fitted to single- or bi-exponential decay functions in SAAM II (SAAM Institute, Seattle, WA). Data were fitted with a fractional standard deviation of 0.05, using central derivative and relative weighting based on the model. Obvious outliners were excluded from the fitting process.

Statistics

Data were analyzed for statistical significance by independent samples *t*-test using the SPSS program (SPSS Inc., Chicago, IL) for Windows. A *P* value less than 0.05 was considered significant and is indicated by *. The data are presented as means ± SD unless otherwise specified.

RESULTS

LPL in normal mink and effects of fasting

Table 1 shows lipase activity and LPL mass in fed and fasted normal mink. From studies in other animal species it was expected that fasting should decrease LPL activity in adipose tissue (8–10). When mink were fasted overnight, LPL activity and mass decreased to about half of fed values in subcutaneous and abdominal fat. The difference was,

TABLE 1. Lipase activity and LPL mass in tissue homogenates from fed and fasted mink

Tissue	Lipase Activity		LPL Mass	
	Fed	Fasted	Fed	Fasted
	U/g		*μg/g*	
Abdominal fat	1.02 ± 0.54	0.45 ± 0.31	8.60 ± 3.70	3.75 ± 1.04[a]
Subcutaneous fat	0.85 ± 0.48	0.53 ± 0.10	12.2 ± 5.1	6.31 ± 0.77[a]
Perirenal fat	0.95 ± 0.56	0.98 ± 0.28	11.5 ± 5.0	12.1 ± 1.9
Heart	1.53 ± 0.43	1.31 ± 0.39	25.8 ± 10.6	15.9 ± 3.4
Quadriceps muscle	0.78 ± 0.59	1.21 ± 1.32	9.05 ± 4.52	8.66 ± 2.88
Diaphragm	0.28 ± 0.12	0.18 ± 0.08	4.37 ± 0.86	3.47 ± 1.15
Kidneys	4.30 ± 0.87	3.89 ± 0.74	70.3 ± 15.4	67.4 ± 14.0
Spleen	0.28 ± 0.19	0.12 ± 0.07	4.38 ± 1.65	2.05 ± 0.45
Lungs	0.43 ± 0.25	0.30 ± 0.13	4.30 ± 1.11	2.77 ± 0.78
Liver	0.56 ± 0.09	0.65 ± 0.25	5.30 ± 1.72	4.08 ± 0.43

For this experiment, normal mink weighing 890–2036 g were used. One male and four female mink were fasted overnight (mean weight 1530 ± 440 g); three male and two female mink were not fasted (mean weight 1040 ± 220 g). The mink were anesthetised and labeled chylomicrons were injected. A series of blood samples were taken to follow the disappearance of the labeled material (Fig. 1). After 15 min the animals were killed and tissues were removed for analysis of lipase activity and LPL mass (this table) and tissue distribution of the injected labeled material (Table 2). Data are means ± SD.

[a] $P < 0.05$, fed versus fasted.

however, significant only for LPL mass. There was no change in perirenal fat. LPL activity was high in heart and quadriceps muscle but lower in the diaphragm. There was no statistically significant change after fasting in any of the three muscles. A remarkable finding was a very high lipase activity in the kidney. High renal activity has been found in all normal mink ($n > 20$) that we have analyzed during the 3-year study period. High amounts of LPL mass were also present in kidney homogenates. As in other animal species, there was LPL activity in spleen and lung also. A significant amount of lipase activity was recorded in liver, 0.56–0.65 U/g tissue. This may have been due to hepatic lipase, which is active under our conditions for assay of LPL (26).

The relationship between LPL activity and mass was between 50 and 170 U/mg in the tissues studied. There was no significant difference between fed and fasted mink in any of the tissues. Most of the values were, however, significantly lower than 280 U/mg which was calculated for the active form of mink LPL, as separated by heparin-Sepharose chromatography of post-heparin plasma (26). This indicates that the tissues contain both active and inactive forms of the enzyme in accordance with the situation found also in other animal species.

To study the metabolism of chylomicrons, we used lymph chylomicrons from rats. When [^{14}C]oleic acid-labeled chylomicrons were injected to mink, TG radioactivity disappeared following a bi-exponential curve, where the first phase most likely corresponded to active hydrolysis of TG from the chylomicrons (**Fig. 1**). This initial clearance corresponded to half-times of 1.77 ± 0.34 min and 1.59 ± 0.25 min in fed and fasted mink, respectively. Some radioactivity reappeared in the plasma FFA fraction and reached a maximum corresponding to about 3% of the injected radioactivity in fed and about 5% in fasted mink at 4–5 min after the injection.

The amounts of ^{14}C radioactivity (oleic acid) that accumulated in tissues were measured at 15 min after injection of the labeled chylomicrons (**Table 2**). Adipose tissue contained 0.15% g and 0.26%/g of the injected dose in fed and fasted mink, respectively. The difference was not statistically significant. The weights of the animals varied from 890 g to 2040 g and there was a large difference in the amount of adipose tissue between them. Assuming that there was an average 200 g adipose tissue per mink, the uptake corresponded to 30–50% of the injected dose. In the heart, about 2% of the injected dose was recovered. Quadriceps muscle contained 0.1–0.2% of the injected radioactivity per g tissue. If muscles make up half of the body weight, and all muscles took up as much chylomicron lipids as the quadriceps did, that uptake would correspond to more than the injected dose. We have not measured uptake by other muscles, but the data indicate that a large part of the fatty acids from chylomicron TG are deposited in muscle. Almost no radioactivity was found in

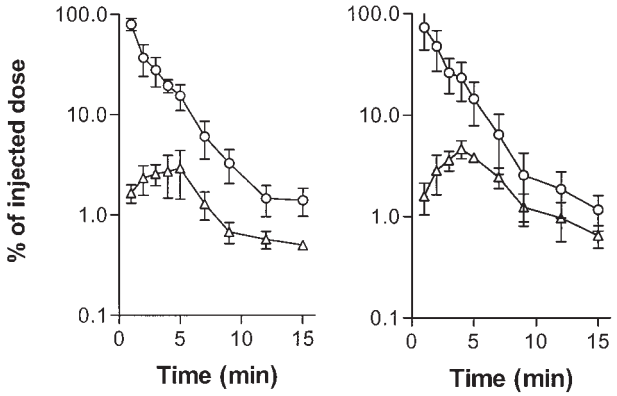

Fig. 1. Clearance of TG-labeled chylomicrons in normal mink. Chylomicrons obtained from a donor rat fed [1-^{14}C]oleic acid (incorporated in chylomicron TG) were injected intravenously in fed (left panel) and fasted (right panel) anesthetized mink, same animals as in Tables 1 and 2; ○, label in TG and other fatty acid esters; △, label in FFA. Values are percent of injected dose present in the total blood volume (calculated as 7.8% of body weight), mean of five in each group ± SD.

TABLE 2. Tissue distribution of radioactivity after injection of TG labeled chylomicrons

Tissue	^{14}C Radioactivity	
	Fed	Fasted
Subcutaneous fat, per g	0.15 ± 0.08	0.26 ± 0.08
Heart, per organ	1.64 ± 0.65	2.06 ± 0.32
Quadriceps muscle, per g	0.10 ± 0.05	0.19 ± 0.04[a]
Kidneys, per organ	0.24 ± 0.04	0.30 ± 0.16
Spleen, per organ	1.75 ± 0.83	1.90 ± 0.68
Lungs, per organ	0.92 ± 0.44	0.84 ± 0.36
Liver, per organ	10.0 ± 2.0	9.36 ± 1.86

Same experiment as in Table 1 and Fig. 1. The animals were killed 15 min after injection of the labeled chylomicrons. Values are percent of injected dose ± SD per organ or per g tissue, n = 5.

[a] $P < 0.05$, fed versus fasted.

the kidneys, although high LPL activity was present there. About 2% of the radioactivity was found in spleen and about 1% in lungs. In liver, 10.0% and 9.4% of the radioactivity was recovered in the fed and fasted states, respectively. Altogether there were no significant differences between fed and fasted mink except for the quadriceps muscle, where the difference was of borderline significance. This information was necessary as background for the studies on hypertriglyceridemic mink described below.

Hypertriglyceridemic mink

During the first year of our experiments (in 1995) two homozygous kits were identified by having turbid plasma. Together with four normal mink (**Table 3**, group A) the two deficient mink were kept on a fat-reduced diet for about 10 days to lower plasma TG. At 14 weeks of age, they were fasted overnight and the experiments were done. Plasma TG levels were 12 and 83 mmol/l in the two hypertriglyceridemic mink compared to a mean value of 2.6 mmol/l in two normal mink (Table 3, group A). Plasma cholesterol was elevated to 12 and 22 mmol/l compared to a mean of 4.6 mmol/l in the two normal mink. The next year, three homozygote kits and seven normal kits, all fed a high fat diet (normal mink diet), were used in our experiments at 10 weeks of age (Table 3, group B). They had much higher levels of plasma lipids compared to the mink used in group A, 166–226 mmol TG/l and 22–31 mmol cholesterol/l, respectively.

Animals homozygote for the LPL mutation (Table 3, group B) had traces of lipase activity in all tissues studied. Values from 2.3% to 12% of those in normal mink were recorded (**Table 4**). Measurements of LPL mass showed that both normal and deficient mink had LPL protein in all tissues examined. Homozygote mink had significantly more LPL protein in muscle and adipose tissue than normal mink. In the heart the amounts were similar. Homozygote mink had less LPL protein in kidney, but the difference was not statistically significant. In an attempt to further characterize the low lipase activity in tissues of hypertriglyceridemic mink, we combined all available frozen tissue (1.3–2.9 g of kidney, adipose tissue, and heart), made homogenates, and chromatographed the homogenate on heparin-Sepharose. For kidney, a clear peak of LPL activity was eluted by the salt gradient in a position expected for active LPL dimers. The activity corresponded to about 4% of the activity recovered from an equivalent amount of adipose tissue from a normal mink, which also corresponded well to the activity in the tissue homogenate before chromatography. A small peak of LPL mass was recorded in the corresponding fractions, indicating that a minor part of the mutated protein was able to form active dimers. The main part of LPL mass eluted in the position for inactive LPL. A similar pattern was seen with homogenate from heart, whereas the pattern for adipose tissue was not well resolved. The specific activities of the peaks of LPL from kidney and heart were lower than that from adipose tissue from a normal mink (2.4 and 5.3 U/mg, respectively, compared to more than 100 U/mg for LPL from normal adipose tissue). These results indicate that the hypertriglyceridemic mink have small amounts of active LPL in their tissues, with normal affinity for heparin, but with markedly decreased specific activity. This lipase is

TABLE 3. General characteristics and plasma lipids of the normal and hypertriglyceridemic mink studied, and the fractional catabolic rates for core and TG label from injected chylomicrons

Group	Type	n	Weight	Plasma Lipids		FCR	
				Chol	TG	Core Label	TG Label
			g	*mmol/l*		*pools/min*	
A: Fasted							
Female	Normal	4	829 ± 116	4.6 ± 1.4	2.6 ± 1.2	0.068 ± 0.023	0.33 ± 0.22
	LPL-deficient	2	780, 798	12, 22	12, 83	0.014, 0.009	0.013, 0.011
B: Fed							
Female	Normal	4	908 ± 130	6.3 ± 0.4	1.7 ± 0.4	0.070 ± 0.028	0.44 ± 0.14
	LPL-deficient	2	560, 585	30.7, 22.3	166, 196	0.019, 0.018	0.019, 0.019
Male	Normal	3	1289 ± 242	5.7 ± 0.9	1.6 ± 0.7	0.047 ± 0.023	0.32 ± 0.06
	LPL-deficient	1	1231	26.3	226	0.004	0.007

The body weights and plasma lipid concentrations are for the day of the experiment, i.e., in the fasted state, for group A (14-week-old mink) and in the fed state for group B (10 weeks old). The fractional catabolic rates were calculated assuming that the disappearance followed a single exponential during the entire 30-min time period for the core label, and biexponential decay for the TG label as illustrated in Fig. 2; FCR, fractional catabolic rate; n, number of animals in each group. In group A the TG and Chol levels were determined in only two of the control minks. Values given as means ± SD.

TABLE 4. Lipase activity and LPL mass in tissue homogenates from normal and from hypertriglyceridemic mink

Tissue	Lipase Activity		LPL Mass	
	Normal	LPL-Deficient	Normal	LPL-Deficient
	U/g		*μg/g*	
Abdominal fat	1.50 ± 0.20	0.18 ± 0.13[a]	12.6 ± 2.6	37.7 ± 11.1[a]
Heart	1.52 ± 0.49	0.10 ± 0.01[a]	27.9 ± 13.6	34.2 ± 6.7
Quadriceps muscle	1.13 ± 1.98	0.08 ± 0.12	9.83 ± 2.04	17.1 ± 9.0[a]
Kidneys	6.05 ± 2.03	0.14 ± 0.03[a]	118 ± 77	30.8 ± 4.7
Liver			5.85 ± 2.76	7.41 ± 3.63

Seven normal and three hypertriglyceridemic mink (group B, Table 3) were anesthetized and doubly labeled chylomicrons were injected. Blood samples were withdrawn (data not shown) and after 40 min the animals were killed. Tissues were removed for analysis of lipase activity and LPL mass (this table) and tissue distribution of the injected labeled material. Values are given as means ± SD.

[a] $P < 0.05$, normal versus hypertriglyceridemic mink.

probably not secreted to the endothelium, as no active LPL was previously found in post-heparin plasma (26).

Doubly labeled ([^{3}H]retinol, core label and [^{14}C]oleic acid, TG label), rat lymph chylomicrons were injected in the eleven normal and five hypertriglyceridemic mink listed in Table 3 (**Fig. 2**). Normal mink cleared chylomicron constituents rapidly (see also Fig. 1). Fifteen min after injection, 19 ± 4% of the core and 1.9 ± 0.2% of the TG label remained in blood (mean values for the four normal mink in group A). The effect of lipolysis was extensive as evidenced by the more rapid clearance of TG label compared to core label and by the appearance of ^{14}C radioactivity in the plasma FFA (**Fig. 3**). Radioactivity in FFA reached a maximum of about 2% of the injected dose at 3 min in normal mink. The hypertriglyceridemic mink cleared core and TG label much slower (Fig. 2) and there was no initial increase of radioactivity in the FFA fraction (Fig. 3). After 15 min, 71% and 74% of core and TG label, respectively, remained in the circulation (mean of the two hypertriglyceridemic mink in group A). Figure 2 shows data from one of the two. Table 3 shows values for fractional catabolic rates (FCR) calculated from data for both groups of animals studied. The mean FCR (pools per min) ranged between 0.047 and 0.070 for core label and between 0.32 and 0.44 for TG-label in the normal animals (Table 3). In hypertriglyceridemic mink, values for FCR were on the average 5-fold lower for core label and 26-fold lower for TG label. Assuming, however, that most of the endogenous TG-rich lipoproteins were turned over at rates similar to those for the injected chylomicrons, the total turnover in the two hypertriglyceridemic, fasted mink in group A was 29% and 169% of the TG turnover in the two normal animals for which the level of plasma TG was known. In the fed ani-

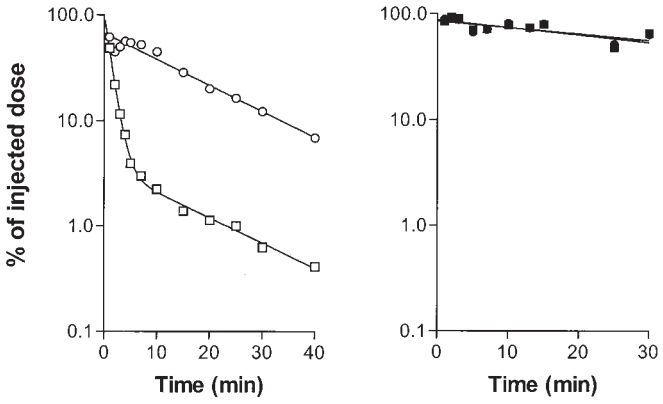

Fig. 2. Clearance of chylomicrons in hypertriglyceridemic mink. Doubly labeled chylomicrons were injected intravenously in eleven normal and five hypertriglyceridemic mink (Table 3). The left panel shows data for one representative of the normal mink (open symbols); the right panel shows data for one of the hypertriglyceridemic mink (solid symbols). Values are percent of injected dose present in the total blood volume (calculated as 7.8% of body weight). The lines are from regression analysis assuming that the disappearance followed a single exponential during the entire 30-min period for the core label in both animals and for TG label in the hypertriglyceridemic mink, while the clearance of TG label in normal mink was biexponential; □, ■, TG label; ○, ●, core label. The appearance of label in plasma FFA in this experiment is shown in Fig. 3; the tissue distribution of label at 30 min is given in Table 5.

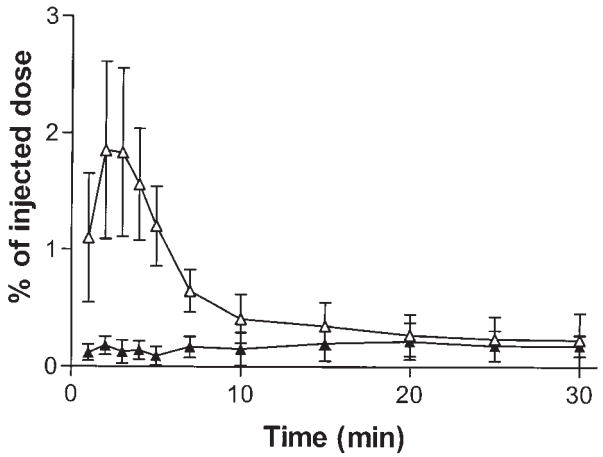

Fig. 3. Appearance of label in plasma FFA after injection of labeled chylomicrons to normal and hypertriglyceridemic mink. Same experiment as in Fig. 2. Values are means ± SD of injected TG label in 11 normal mink (△) and 5 hypertriglyceridemic mink (▲). The values were significantly different, LPL hypertriglyceridemic versus normal, at all times up to 10 min. Note that the y scale is linear here in contrast to that shown in Figs. 1 and 2 where the scale is logarithmic.

mals (group B), hypertriglyceridemic mink turned over more TG than did the normal mink (on average 430% of the turnover in the group of seven normal animals).

To compare the degree of lipolysis of the chylomicrons remaining in plasma, we calculated a lipolysis index (TG label/core label) for each sample. An index less than one indicates that the particles had lost TG. In the normal mink, the lipolysis index had decreased to about 0.8 already at 2 min and then decreased to about 0.1 during the following 8 min (Table 3 group A, and Fig. 2). In the hypertriglyceridemic mink of group A, the lipolysis index did not change significantly (1.1–0.8) during the observed time. The lipolysis indexes for the fed animals in group B (Table 3) were similar to those in group A. In the normal mink, the index had decreased to about 0.6 already by 2 min and then decreased to 0.06 during the following 8 min. In the hypertriglyceridemic mink, the mean lipolysis index was between 0.8 and 1.0 during the whole experiment. Another finding supporting the observation that there was no intravascular lipolysis of chylomicron TG in the hypertriglyceridemic mink was that almost no radioactivity appeared in the plasma FFA at early times (Fig. 3). Only after 15–20 min was a small increase seen.

To study how chylomicrons were distributed to the respective tissues, we measured core radioactivity in tissue homogenates. In the liver, 58.0 ± 9.2% of core label was found after 30 min in the normal mink. This was significantly more than the 36.4 ± 6.4% found in livers of the hypertriglyceridemic mink (**Table 5**). Calculated ratios of TG/core (lipolysis index) indicated that livers of normal mink took up extensively lipolyzed chylomicrons (index = 0.1 ± 0.1). In contrast, the index in livers from the hypertriglyceridemic mink was 1.1 ± 0.2, suggesting removal of largely nonlipolyzed chylomicrons. Adipose tissue and also muscle and heart in the normal mink accumulated more fatty acid than retinol label, suggesting preferential uptake of fatty acids released by lipolysis of chylomicron TG. This was not the case in the hypertriglyceridemic mink where the ratio was close to one.

From the tissue distribution of label as listed in Table 5 it was difficult to directly compare the contribution of each tissue to the removal of chylomicron constituents, as quite different amounts of label remained in the circulating blood in the two groups of animals. Therefore we have recalculated the data for each animal, in terms of percent of label that had left blood. Data from two experiments (Table 3, groups A and B) were pooled. When expressed in this way, 40–60% of the cleared core label was found in livers of both groups of animals (**Fig. 4**). For the other tissues studied, there were no significant differences in the fraction of cleared core label. For instance, about 1% was in the heart and 0.1% in abdominal fat/g or quadriceps muscle/g of both normal and hypertriglyceridemic mink. In normal mink, 4.0 ± 0.5% of the cleared core label was found in the spleen, compared to 2.0 ± 1.7% in spleen of hypertriglyceridemic mink. In contrast, there were striking differences for the TG label. There was much more of this label in the liver of hypertriglyceridemic animals compared to normal mink (Fig. 4) and consequently less in peripheral tissues. About 2.3% was in the hearts of normal mink compared to 0.8% in hypertriglyceridemic mink. In spleen the results resembled those in liver but at a lower level (1.9 ± 2.5% and 0.5 ± 0.4% of TG label in hypertriglyceridemic and normal mink, respectively), indicating that binding/uptake was mainly of chylomicrons (remnants or unlipolyzed) rather than of FFA, which is also evident from the data in Table 5 (compare liver, heart, and spleen).

Rapidly floating, TG-rich lipoproteins (named chylomicrons and light VLDL, respectively), as well as the normal VLDL fraction, were isolated from fresh mink plasma (two animals in each group, all fasted >12 h). The amount of VLDL-TG recovered from the normal mink was very low. The total TG recovered in the three fractions from the hypertriglyceridemic mink was approximately distributed in the ratios 50:2:1 for chylomicrons: light VLDL: VLDL. SDS-PAGE and Western blots

TABLE 5. Distribution of label in tissue after injection of doubly labeled chylomicrons to normal and LPL-deficient mink

	Normal			LPL-Deficient		
Tissue	Core Label	TG Label	Ratio TG/Core	Core Label	TG Label	Ratio TG/Core
Abdominal fat, per g	0.12 ± 0.02	0.31 ± 0.31	2.8 ± 3.1	0.06 ± 0.02	0.04 ± 0.02	0.7 ± 0.1
Subcutaneous fat, per g	0.15 ± 0.07	0.36 ± 0.36	2.3 ± 1.8	0.09 ± 0.02	0.05 ± 0.01	0.6 ± 0.1
Heart, per organ	1.44 ± 1.92	2.74 ± 1.57	3.6 ± 2.4	0.65 ± 0.24	1.02 ± 0.59	1.6 ± 0.7
Quadriceps muscle, per g	0.13 ± 0.07	0.15 ± 0.19	1.3 ± 1.1	0.07 ± 0.03	0.05 ± 0.03	0.7 ± 0.4
Spleen, per organ	3.63 ± 2.21	0.67 ± 0.21	0.2 ± 0.1	1.04 ± 0.16	0.67 ± 0.11	0.7 ± 0.1[a]
Lungs, per organ	2.30 ± 1.93	0.80 ± 0.22	0.5 ± 0.2	1.15 ± 0.56	0.99 ± 0.36	0.9 ± 0.1[a]
Kidneys, per organ	0.73 ± 0.93	0.12 ± 0.04	0.3 ± 0.2	0.33 ± 0.25	0.24 ± 0.18	0.7 ± 0.3[a]
Liver, per organ	58.0 ± 9.2	6.3 ± 1.1	0.1 ± 0.1	36.4 ± 6.4[a]	38.3 ± 1.4[a]	1.1 ± 0.2[a]

At the end of the experiment shown in Fig. 2, the animals were killed and tissue samples were taken. Data shown are tissues from group B, Table 3. Values are percent of injected dose. A ratio of TG label/core label, "lipolysis index," of one indicates equal amounts of the respective labels in the tissue. Indexes lower than one suggest uptake of lipolyzed particles, whereas a number above one suggests preferential uptake of fatty acids. Values given as means ± SD.

[a] $P < 0.05$, homozygote versus normal.

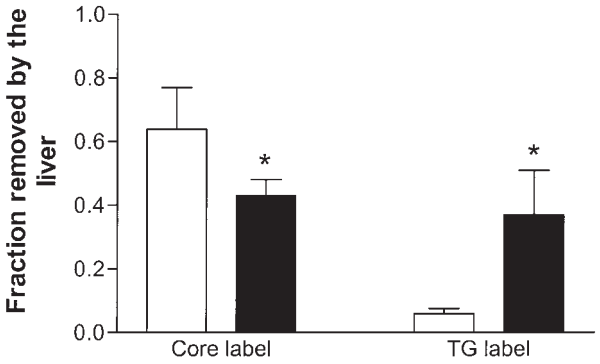

Fig. 4. Net recovery of label in liver. This figure gives combined data from group A and B (Table 3), hence values are mean for 11 normal (open bars) and 5 hypertriglyceridemic (filled bars) mink. Note that the tissues from group A were removed 30 min after injection of labeled chylomicrons whereas for group B they were removed after 40 min. The total amount of label cleared from blood was calculated as 100% minus that remaining in blood at the last time point. The fraction of this that had been removed by the liver was then calculated as follows. First, the contribution of radioactivity from blood present in the liver was subtracted from the total radioactivity in the liver; then, the difference was divided by the total amount cleared from blood.

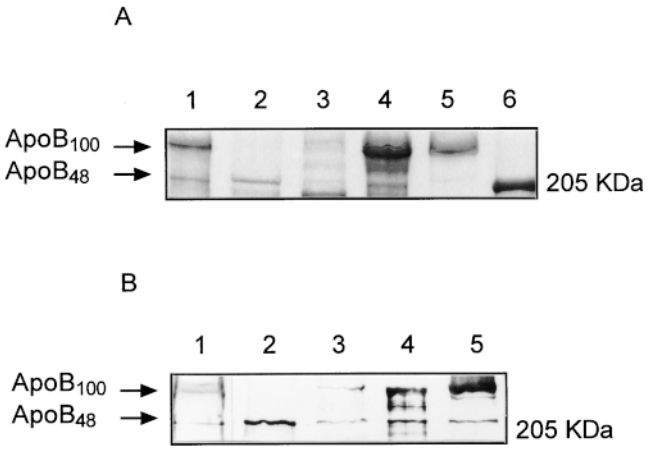

Fig. 5. SDS-PAGE of delipidated large TG-rich lipoproteins and of VLDL. Panel A shows a gel stained with Coomassie blue. Lane 1, human VLDL (5 μg protein); lane 2, chylomicrons from hypertriglyceridemic mink (15 μg); lane 3, light VLDL from hypertriglyceridemic mink (15 μg); lane 4, VLDL from hypertriglyceridemic (15 μg); lane 5, VLDL from normal mink (15 μg); and lane 6, high range molecular weight standard. Panel B shows a Western blot with antibodies against human apoB. Lanes 1–5 are the same as in panel A but the amounts of protein applied per lane were 1 μg, 5 μg, 5 μg, 1 μg, and 5 μg, respectively. The lipoprotein fractions were isolated as described in the Methods section.

showed that apolipoprotein B-100 (apoB-100) was dominant in the VLDL fraction from both groups of animals. ApoB-48 was dominant in the chylomicron fraction from the hypertriglyceridemic mink while the light VLDL fraction contained a mixture of both apoB-100 and apoB-48 (**Fig. 5**). Thus, most of the TG-rich lipoproteins in the hypertriglyceridemic mink were probably of intestinal origin.

DISCUSSION

LPL hypertriglyceridemic, mutant mink offered an animal model to study, for the first time, how chylomicrons in blood are handled in the absence of LPL. The results reveal that at least two factors contribute to the problems encountered by the animals. One is a much slower fractional catabolism of the TG, but another factor is that because TG are not unloaded from the chylomicrons by LPL in peripheral tissues, they follow the particles to the liver. There they might stimulate a futile cycle of TG transport: out from the liver in the form of VLDL and return to the liver in unlipolyzed VLDL. All hypertriglyceridemic mink used here had enlarged fatty livers weighing 1.8- to 3.2-fold more than livers from corresponding control animals. Also, the spleens were heavier in the LPL hypertriglyceridemic mink (3.9- to 5.5-fold). In older animals, severe lipid granulomas develop as previously described by Christophersen et al. (26).

Initial studies of normal mink showed that LPL activity was distributed among their tissues in a pattern similar to that in rats, mice, and guinea pigs with two exceptions (8, 9, 18, 19). One was that mink had very high LPL activity and mass in kidneys, even higher than in adipose tissue. We are not aware of such high LPL activity in kidneys of any other animal species. In rats, mice, and guinea pigs, the LPL activity is low in the kidneys. Camps et al. (33) found intense LPL immunoreactivity in glomeruli of guinea pig kidneys. There were no nearby cells that contained LPL mRNA, as studied by in situ hybridization. Therefore, these authors suggested that the lipase was picked up from blood by the abundant heparan sulfate proteoglycans in the glomeruli (33). The cellular localization and function of LPL in mink kidneys is presently unknown. Only trace amounts of labeled lipids from the injected chylomicrons went to the kidney. Hence, if kidney LPL is located so that it can reach plasma lipoproteins, the enzyme must release fatty acids mainly into the circulating FFA pool.

The other difference from other animal species was that fasting for 12 h did not cause any significant decrease in the LPL activity in mink adipose tissue, in contrast to the 5- to 20-fold drop in the activity that occurs with fasting in rats or guinea pigs (8, 9). We have not studied what happens when mink are fasted for a longer time, but it is clear that the acute response is less pronounced than in other animal species. The ratio of LPL activity to LPL mass was 50–170 U/mg in the mink tissues. This is significantly less than the value of 280 U/mg recorded for the active species of LPL, as isolated from post-heparin plasma by chromatography on heparin-Sepharose (26), and suggests that mink tissues contain both active and inactive forms of the lipase similar to the situation in rats (9). On chromatography of a homogenate from normal mink adipose tissue (not fasted) on heparin-Sepharose, there was 1.4-fold more LPL protein in the peak without activity and with lower heparin affinity than in the peak of active LPL

with high heparin affinity (data not shown). In adipose tissue from fed rats, the corresponding peak ratio is 0.46. In fasted rats it increased to 2.34 (9). Thus, in mink adipose tissue, the ratio was somewhere between the fasted and the fed states in rats.

Christophersen et al. (26) found, by separation on heparin-Sepharose, that the lipase activity in post-heparin plasma from hypertriglyceridemic mink was due entirely to hepatic lipase. There was no measurable LPL activity. There was, however, inactive LPL protein in plasma. This LPL was only released by heparin to a minor extent, indicating that it was mostly circulating, presumably as monomers with low heparin affinity (34). In the present study we found traces of lipase activity in tissue homogenates from hypertriglyceridemic mink homozygous for the mutation in the LPL gene. For instance, in adipose tissue the lipase activity was 0.18 U/g tissue in hypertriglyceridemic mink compared to 1.50 U/g in the normal mink. The levels of LPL mass were either increased (in abdominal fat and quadriceps muscle) or similar (in heart and kidneys) compared to those in controls. On studies of heterozygotes for the mutation, Lindberg et al. (27) found increased levels of both LPL protein and mRNA in heart and adipose tissue, while LPL activity was reduced to about 50% of the levels in normal animals. Thus the Pro214Leu mutation probably results in production of mostly catalytically incompetent protein, but some compensatory mechanism may exist to increase transcription of the LPL gene in muscle and adipose tissue of the affected animals. In the present study we made an attempt to characterize the low lipase activity found in the tissues of the hypertriglyceridemic animals. We found that some LPL activity eluted from heparin-Sepharose in the position expected for normal, catalytically active, dimeric LPL. This was accompanied by a small peak of immunoreactive LPL protein, although the majority of the LPL protein eluted in the position of inactive, probably monomeric, LPL. The specific activity of the LPL eluted from "LPL-deficient" kidneys and heart amounted to only a few percent of that recorded for LPL eluted from normal mink adipose tissue. Although preliminary, these data indicate that the mutation impeded formation of active LPL dimers, but that a small fraction of LPL still succeeded in forming dimers with some catalytic capability. As no activity was previously found in post-heparin plasma (26), the mutated LPL dimers are presumably not transported to the vascular endothelium and will therefore not contribute to lipolysis of blood lipids to any significant extent. It cannot be ruled out, however, that the reason why the homozygote mink survive the postnatal stage, while LPL-deficient mice invariably die, is that the mink have traces of functional LPL.

Normal mink cleared chylomicrons rapidly. The clearance of TG was faster in mink (FCR = 0.31–0.61 min^{-1}) than in rats in similar experiments, where the FCR was 0.158 min^{-1} (5). The ratio of TG to core label in plasma decreased to 0.8 already by 2 min and to about 0.1 within 10 min. Hence, chylomicrons are efficiently depleted from TG in mink, presumably mediated by LPL. In comparison, in rats, this ratio was over 0.9 at 2 min and around 0.5 at 10 min (5). Another indication of rapid lipolysis of chylomicron TG in mink was the early appearance of labeled FFA. The capacity for efficient lipolysis is in accord with the observation that normal mink have very low levels of TG-rich lipoproteins or VLDL in blood (26). In fact, most of the triglycerides are in LDL-sized particles.

Hypertriglyceridemic mink cleared the injected chylomicrons at a much slower rate. There was no indication that lipolysis occurred; the ratio between TG and core label did not change significantly and the initial rise of label in plasma FFA was not present. These data clearly show that hepatic lipase, which is present in normal amounts in the hypertriglyceridemic animals (26), was not able to substitute for LPL to any significant extent. The data also demonstrate that if any partially functional LPL was present at the vessel walls in homozygote mink, the amounts were insufficient.

Even though the clearance of injected chylomicrons was slow in the hypertriglyceridemic mink, there was in each individual a significant slope for clearance of plasma radioactivity with FCRs of 0.004–0.019 min^{-1}. There were large amounts of endogenous TG-rich lipoproteins in plasma (about 200 mmol TG/l). If one assumes that most of these were turned over at rates similar to those for the injected chylomicrons, the overall plasma turnover of TG becomes quite significant, 1.8–7.6 g per h and kg body weight.

The mechanism by which the chylomicrons were cleared in the hypertriglyceridemic mink is not clear, but likely involved endocytosis and delivery to lysosomes. This is in accord with the appearance of some label in the plasma FFA after 15 min, and contrasts to the normal mink where label appeared in the FFA within the first minutes, presumably from lipolysis at endothelial sites. When this does not happen, and the particles have to be transported into cells for degradation in endosomes or lysosomes, one expects that less label would leak back into plasma and that this would occur at later times, as observed. We cannot determine from the present data whether inactive LPL has any role in the clearance. Inactive LPL was present in hypertriglyceridemic mink plasma in about similar amounts as in normal mink plasma (26).

In normal mink most of the fatty acids from the chylomicron TG were deposited in extrahepatic tissues. Only about 10% of the fatty acid radioactivity but about 60% of the core label was in the liver at 30 min, in accord with the view that the liver takes up mainly TG-depleted remnant particles (35). In a balanced situation, the amount of chylomicron fatty acids taken up by the liver may correspond to the energy demand of the liver itself. In functionally LPL-deficient mink, the situation is out of balance. The proportion of the core label that located in the liver was similar to that in normal mink, about 40% of cleared label, but an equal proportion of the chylomicron fatty acids followed the particles into the liver as there was no extrahepatic lipolysis. A large excess of fatty acids above the energy requirement of the liver was created leading to visible accumulation of lipids in this organ. This excess must be transported away and hence drives production of

large VLDL. This could result in a futile cycle of TG secreted by the liver and returning to the liver which fuels a continuous hypertriglyceridemia. Still, even in the fasted state, most of the TG in plasma was in apoB-48-containing, rapidly floating particles, indicating that they were most likely of intestinal origin.

The nonspecific uptake of unlipolyzed chylomicrons in extrahepatic tissues may provide an adequate but slow supply of lipids, and therefore no severe symptoms of inadequate energy metabolism, e.g., in muscle and heart, are evident in the affected mink. Problems arise from tissues that cannot oxidize the fatty acids or utilize them for synthetic processes. This might be the cause for the accumulation of lipid in macrophages in the abdominal cavity (26). The situation is illustrated in the spleen. The amount of core label removed by the spleen was less than 5% and did not differ between normal and hypertriglyceridemic mink. In normal mink chylomicrons were heavily lipolyzed, whereas in LPL-deficient mink they were not lipolyzed at all, leading to undesired TG accumulation in this organ.

In summary, functional LPL deficiency in mink severely impedes chylomicron metabolism. No lipolysis occurs, illustrating that hepatic lipase is unable to compensate for the LPL deficiency. Core and TG label disappeared from blood with the same slow rate indicating removal of essentially unlipolyzed chylomicrons. Tissue distribution was similar and net uptake of label was not affected by LPL deficiency with respect to core label, whereas the lack of TG depletion of chylomicrons resulted in increased uptake of TG in some tissues (liver and spleen) of affected animals. Which mechanisms, receptor-dependent or receptor-independent, are involved in the removal of the chylomicrons remains to be elucidated.

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