Lipoprotein lipase deficiency with pancreatitis in mink: biochemical characterization and pathology

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Abstract A severe hyperlipemia in mink, with a pattern that suggested recessive inheritance, was observed at a farm in Norway. On a normal mink diet, affected animals had grossly elevated levels of plasma triglycerides which decreased towards normal on a low-fat diet. Normal minks had the main part of their plasma cholesterol in the HDL fraction. Affected minks, although severely hypertriglyceridaemic, had almost normal levels of both LDL and HDL. Affected minks frequently had lipogranulomas in the mesentery and the pancreas. The lipogranulomatous tissue contained spaces filled with an amorphous, sudanophilic substance with many foamy macrophages in the fibrous tissue between the lesions. Separation of post-heparin plasma on heparin-agarose revealed that the affected minks had no detectable lipoprotein lipase activity but normal activity of hepatic lipase. Both normal and affected minks had inactive lipoprotein lipase protein in pre- and post-heparin plasma. This protein, which eluted before the active lipase from heparin-agarose, probably corresponds to lipase monomers. The presence of lipoprotein lipase mass in the affected minks, but no activity, indicates that there might be a point mutation in the lipase gene. The minks provide a new animal model for studies on pancreatitis induced by hypertriglyceridemia and on lipoprotein metabolism in the lipoprotein lipase-deficient state and show features similar to those found in human hyperlipoproteinemia type I.—**Christophersen, B., K. Nordstoga, Y. Shen, T. Olivecrona, and G. Olivecrona.** Lipoprotein lipase deficiency with pancreatitis in mink: biochemical characterization and pathology. *J. Lipid Res.* 1997. **38:** 837–846.

Supplementary key words Type I hyperlipoproteinemia • heparin-Sepharose • lipase monomer • hepatic lipase

Hyperlipoproteinemia Type I is a rare inborn error of metabolism due to deficiency of functional lipoprotein lipase (LPL) or its activator apolipoprotein C-II (apoC-II) (1–3). In humans the clinical picture includes massive accumulation of chylomicrons in plasma, low levels of LDL and HDL, eruptive xanthomas, lipemia retinalis, hepatosplenomegaly, recurrent attacks of abdominal pain and of pancreatitis (1–3). The most severe problem is attacks of pancreatitis that occur when the levels of plasma triglycerides are high (3) in LPL deficiency as well as in other types of hyperlipidemia. The cause for the involvement of the pancreas has not yet been elucidated. Studies of the development of the pancreatitis lesions in humans is complicated by the fact that abdominal surgery is contraindicated in these patients and also because this organ is subject to a very rapid autolysis post-mortem.

In apoC-II deficiency the plasma lipid levels can be normalized for weeks by transfusion with plasma from a healthy donor who provides the lacking activator (4). For LPL deficiency there is as yet no treatment, apart from a strict low-fat diet supplemented with fat-soluble vitamins and essential fatty acids and sometimes medium-chain fatty acids. The patients are candidates for trials with somatic gene therapy (5). Many of them are detected in childhood but their clinical manifestations may vary from mild to severe chylomicronemia. There are LPL- or apoC-II-deficient individuals who have lived without knowing the cause of their aversion for lipid-rich food until adulthood.

Type I patients have not been considered to develop excessive atherosclerosis, although their vessel walls are exposed to very high levels of abnormal lipoproteins for many years. Zilversmit (6, 7) proposed that LPL is necessary to transform non-atherogenic large lipoproteins to smaller, atherogenic remnant particles that penetrate more effectively into the arterial wall. This theory has been amended in recent years with suggestions that LPL may serve as a ligand that promotes uptake of lipoproteins by cells of the vessel walls (8). Direct studies

Abbreviations: LPL, lipoprotein lipase; apoC-II, apolipoprotein C-II.

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have not been possible due to the lack of animal models, and it is not known how the triglyceride-rich lipoproteins are metabolized in the absence of functional LPL.

The only known animal model for LPL deficiency is a strain of cats which were recently reported to carry a point mutation in residue 412 resulting in non-catalytic LPL (9–13). The pathologic lesions in cats are somewhat different from those in humans in that the animals develop severe neurological symptoms but no pancreatitis. It is known from several laboratories that mice in which the LPL gene has been targeted do not survive (14). The embryos appear to develop normally in utero, and the newborn mice look normal. When they start to suckle, their blood lipids quickly rise to very high levels. After some hours the pups become cyanotic and die, probably due to suffocation. A similar syndrome is seen in mice carrying the cld mutation (15,16). Cld/cld homozygotes lack both LPL and the related hepatic lipase, presumably due to a defect in the intracellular maturation and transport of the enzymes (17). Heterozygotes for LPL deficiency are usually asymptomatic. Therefore, neither the knock-out mice nor the cld/cld mice are useful for studies of the consequences of LPL deficiency for lipoprotein metabolism or as animal models for the human disease.

A severe hyperlipemia was observed in some minks that were tested for infectious plasmacytosis in mink farms in southwest Norway (18). In the present report we have studied LPL activity and mass in pre- and post-heparin plasma of the minks and establish that the defect is a lack of LPL activity. From this we have proceeded to explore the pathologic lesions of the disease. The results point to many similarities to the human disease including the development of pancreatitis, and make these minks a promising animal model for studies of the different manifestations of the still poorly understood chylomicronemia syndrome.

MATERIALS AND METHODS

Animals

The animals (Standard mink, *Mustela vison*) were kept in outdoor cages in sheds. Their normal diet provided 28–39 E% protein, 32–55 E% fat, and 18–29 E% carbohydrates depending on the raw materials available to the producers. The minks mate only once a year. Each dam gives birth to 4 kits on the average. The kits are born in May and suckle until the beginning of July. Blood samples were taken in EDTA tubes either after killing or from an exposed jugular vein on anesthetized animals. Anesthesia was with Ketalar (Park Davis) 10 mg/kg and Domitor vet. (Farmos) 0.2 mg/kg. The blood was immediately chilled, and plasma was collected after low-speed centrifugation and immediately analyzed for lipids or frozen at −70°C. To obtain post-heparin plasma, heparin (Leo, 100 IU/kg body weight) was injected in the jugular vein of an anesthetized mink and a blood sample was taken after 10 min. The animals were killed by an overdose of Domitor. The body weights of affected minks were in most cases similar to or slightly lower than normal controls, approximately 1 kg in females and approximately 2 kg in males.

The autopsy material included 18 minks of both sexes (8 females and 10 males) between 4 and 21 months of age. Eight animals were killed immediately before autopsy and samples were taken for microscopy. Ten animals were found dead in their cages at feeding time and frozen at −20°C until autopsy could be performed.

The use of experimental animals described in this study has been approved by the local responsible laboratory animal science specialist under the surveillance of the Norwegian Experimental Animal Board and registered by the Board. The experiments have been conducted in accordance with the laws and regulations controlling experiments in live animals in Norway.

Plasma lipid analyses

Triglycerides and cholesterol were analyzed by enzymatic methods (Boehringer Mannheim). HDL cholesterol was determined after precipitation of apoB-containing lipoproteins by $MnCl_2$ and heparin or in one case after ultracentrifugation (19). Agarose gel electrophoreses were run on EDTA plasma on Hydragel Lipo + Lp[a] from Sebia (Issy-les-Moulineaux, France) according to the instructions from the manufacturer. The gel was stained by 0.04% Sudan Black. For analysis of the lipoprotein pattern in normal minks by gel permeation chromatography, 0.5 ml EDTA plasma was applied on a Superose 6 HR 10/30 column (Pharmacia Fine Chemicals, Uppsala, Sweden) which was run at 4°C in 10 mmol/L Na-phosphate buffer, pH 7.4, containing 0.15 mol/L NaCl. Fractions of 0.3 ml were collected and the flow rate was 0.3 ml/min. Plasma from a normal mink fasted overnight (and human plasma for comparison) was first filtered through a 0.22-μm filter. To plasma from the affected minks was added 5 mg EDTA/ml plasma. The plasma was centrifuged for 10 min at 10,000 rpm to remove the main part of the triglyceride-rich lipoproteins which would otherwise have clogged both the filter and the column. The plasma was then filtered through a 0.22-μm filter before chromatography.

Attempts were made to measure plasma amylase activity. Because severe hypertriglyceridemia interferes with

the method used (Boehringer Mannheim), plasma amylase could only be measured in samples with triglyceride concentrations below approximately 16 mmol/L.

Heparin-Sepharose chromatography of plasma samples

Blood samples were taken before and 10 min after intravenous injection of 100 IU heparin/kg body weight. Plasma was applied to small columns of heparin-Sepharose (3 ml gel, 2 ml for pre-heparin plasma) (20) at a flow rate of 0.75 ml/min at 4°C. The columns were then washed with 30 ml 20 mM Tris-HCl, pH 7.4, containing 0.1 M NaCl, 20% (w/v) glycerol, 0.1% (w/v) Triton-X100, and 1 mg bovine serum albumin/ml. The columns were then eluted by gradients of 0.1 to 1.6 M NaCl (70 ml + 70 ml) in the same buffer. The flow rate was 1 ml/min and fractions of 5 ml and 4 ml were collected for post- and pre-heparin plasma, respectively.

Assays for lipase activities

Hepatic lipase was determined using a gum arabic-stabilized emulsion of radiolabeled triolein in incubations at 1 M NaCl, pH 8.5, 25°C (21). In this assay LPL is irreversibly inactivated by the combination of the high salt concentration and the temperature. LPL activity was determined using Intralipid^R 10% (Pharmacia & Upjohn, Stockholm, Sweden), with tritium-labeled triolein incorporated by sonication, as substrate (21). Rat serum was used as source of apoC-II. The concentration of NaCl was 0.1 M. The incubations were carried out at pH 8.5 at 25°C in the presence of 0.1 mg heparin/ml to stabilize LPL (21). One mU corresponds to 1 nmol fatty acid released per min. Hepatic lipase displays partial activity in this assay. For assay of samples from humans, rats, or mice, we use antibodies to suppress hepatic lipase activity. Unfortunately none of our antisera reacted well with hepatic lipase from mink.

Immunoassay for LPL protein

The mink LPL reacted in the enzyme-linked immunoassay (ELISA) previously described for human LPL (22,23). For capture of the antigen on microtiter plates we used affinity-purified antibodies from an antiserum raised against bovine LPL in a chicken (chicken 225). For detection we used the monoclonal anti-bovine LPL antibody 5D2 (a generous gift from Prof. J. Brunzell, Department of Medicine, Washington University Hospital, Seattle, WA) together with a peroxidase-labeled rabbit anti-mouse IgG (A2554, Sigma, St Louis, MO). Human LPL, purified from post-heparin plasma, was used as standard. Dilutions of mink samples reacted reasonably parallel with the human standard curve. We cannot verify that the absolute levels of mink LPL protein are right, but the specific lipase activity of 280 U/mg for mink LPL is similar to values for human and bovine lipases using the same assays.

Light microscopy

Formalin-fixed pieces of organs were embedded in paraffin. Sections were stained with hematoxilin and eosin (HE) and van Gieson. Some frozen sections were stained with Sudan III. Two sections were routinely used for pancreas microscopy until 1993 when the number was increased to 5–6 sections from each gland.

RESULTS

Blood lipids and diets

At weaning (2 months of age) the serum of affected minks appeared white. Plasma lipids were measured at 4–8 months of age on normal and affected minks on a regular diet (**Table 1**). The degree of hyperlipemia varied markedly among the affected individuals, but all were severely hypertriglyceridemic compared to healthy controls. The triglyceride values varied from 13.9 mmol/L in mink 6 to 176 mmol/L in mink 1. Total cholesterol varied from near-normal, 9.8 mmol/L in mink no. 6 to grossly elevated, 39.8 mmol/L, in mink no. 1. **Figure 1a** shows that normal minks have most of their cholesterol in HDL-like particles. In addition, they have varying levels of triglyceride-containing LDL. The animal in Fig. 1a had a fairly high peak of cholesterol corresponding to LDL, while other individuals had only one third of that level. Fasted minks had very low levels of VLDL, which corresponds well to the low mean plasma triglyceride level. Gel permeation chromatography (Fig. 1b) and agarose gel electrophoresis (not shown) of plasma from an affected mink revealed that the levels of LDL and HDL appeared rather normal despite massive hypertriglyceridemia. Most of the increased plasma cholesterol was found in the large, triglyceride-rich lipoproteins. The proportion of triglycerides in LDL was in fact lower in the affected mink than in the normal mink.

The affected animals did not present any visible differences from their littermates. As the animals have to be captured in their cages and given anesthesia before they can be weighed, regular growth curves have as yet not been obtained. At 3 months of age the weight of an affected male was 1.23 kg compared to a mean of 1.28 kg with a range of 1.02 kg–1.50 kg of three control males. The weights of two affected females were 0.59 kg and 0.56 kg compared to a mean of 0.89 kg and a range of 0.75 kg–1.07 kg of 5 control females. At 8 months of age a hypertriglyceridemic male weighed 1.42 kg com-

TABLE 1. Plasma lipid levels in normal and affected minks fed high and low fat diets

Animals	Age	Triglycerides	Total Cholesterol	HDL Cholesterol
	months	*mmol/L*	*mmol/L*	*mmol/L*
Normal controls	4–8 (n = 10)	0.7 ± 0.2	6.3 ± 1.1	5.9 ± 1.0
Affected no. 1	4	176	39.8	
Same animal fed 16 E% fat for 3 weeks	4	11.1	7.0	
Affected no. 2	4	69.6	11.4	
Same animal fed 16 E% fat for 3 weeks	4	4.8	5.3	4.6
Affected no. 3	4	101	19.0	
Affected no. 4	4	27.1	10.0	
Affected no. 5[a]	6	15.2	23.3	
Affected no. 6	8	13.9	9.8	4.5[b]

Animals 1–4 were on the same standard mink diet with approximately 32 E% fat prior to the first blood sampling. After that, two of the affected animals were fed a diet with 16 E% fat for 3 weeks and a second set of blood samples was then taken. Animals 5 and 6 were fed other batches of standard diet but fat contents were not measured.

[a] Affected animal no. 5 had a fatty liver.

[b] HDL cholesterol measured after ultracentrifugation.

pared to a mean of 1.75 kg with range 1.45 kg–1.98 kg of 11 control males.

It was only in older minks that it became apparent that the affected animals did not thrive. Most of them died at ages ranging from 4 to 9 months. Therefore diets with reduced fat contents were tested. A diet with 24 E% from fat appeared to be nutritionally adequate and reduced the hypertriglyceridemia significantly. Three weeks on a diet with 16 E% fat reduced triglycerides from 176 to 11 mmol/L in mink 1 and from around 70 to 4.8 mmol/L in mink 2 (Table 1). Plasma cholesterol decreased into the normal range. On such food the animals failed to thrive and did not gain weight. For technical reasons plasma amylase activities could only be measured in samples with triglyceride concentrations below 16 mmol/L. In the few samples from affected animals where the triglycerides were not higher than this limit, the amylase activities did not differ significantly from those measured in plasma from control minks.

Macroscopic lesions as revealed at autopsy

There was great variation in the autopsy picture of the affected minks, probably because the examined animals differed somewhat in age and had been on different diets. In many cases there were nodular lesions in the jejunal mesentery adjacent to the stomach and duodenum/pancreas with an effect also on the pancreas (Fig. 2). The pancreas was in some cases reduced in size and had an uneven or granular surface. Visible pancreas lesions were found in 7 of the 8 affected animals killed immediately before autopsy. Similar lesions were also present in all 10 affected animals found dead and frozen until autopsy could be performed. In the pancreas of these 10 animals there were additional acute necrotic changes. The pancreas is subject to a very rapid autolysis post mortem, possibly because of activation of exocrine pancreas enzymes. Therefore it was difficult to know with certainty to what extent these acute lesions were the result of post mortem autolysis alone or whether there had also been an acute pancreatitis in vivo. No other obvious cause of death was, however, revealed by autopsy of these animals. It should be noted that it is not unusual in mink farms that some presumably healthy individuals die, and autopsy may not always reveal the cause of death.

The nodular masses in the mesentary consisted of multifocal, coalescing tissue, which was partly necrotic. On the cut surface a semi-liquid, grey-white fluid appeared in some areas (**Fig. 2C**). Other obvious abdominal changes included advanced fatty changes in the liver and a considerable enlargement of the spleen. There were no visible lesions of the peripheral nerves.

Microscopic lesions

Inflammatory lesions were present in the pancreas, with varying proliferation of fibrous tissue, foamy macrophages and corresponding atrophy of pancreas parenchyma (**Fig. 3A**). Such microscopic lesions were found in the pancreas of 17 of the 18 animals studied.

Microscopic examination of the mesenterial lesions showed a lipogranulomatous tissue, with an amorphous, sudanophilic substance within spaces bordered by a flattened cellular layer (Fig. 3B and C). The fibrous tissue between these focal lesions contained many foamy macrophages which sometimes contained hemosiderin (Fig. 3B).

In the liver, fatty changes of varying extent were seen. Lipid laden macrophages were found in many organs, including the spleen, lymph nodes, and bone marrow.

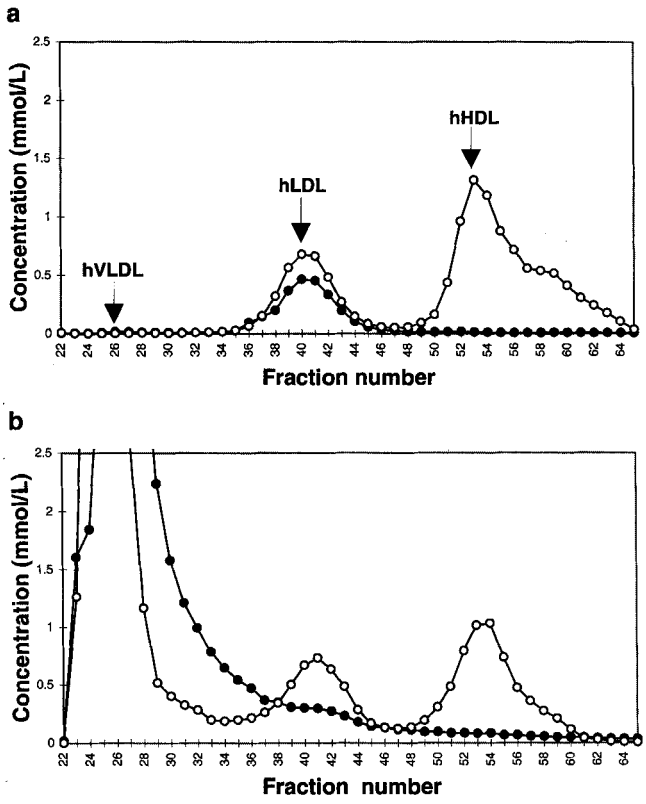

Fig. 1. Lipoprotein profiles in plasma from a normal, fasted mink and from an affected mink were studied by gel permeation chromatography. The chromatography is described in Materials and Methods. Panel a shows the profile from normal mink plasma (triglycerides = 1.22 mmol/L and cholesterol = 7.13 mmol/L) and panel b plasma from a mink with hypertriglyceridemia (triglycerides = 70.9 mmol/L and cholesterol = 17.5 mmol/L). The animals had been fasted overnight before blood sampling; (●) triglycerides; (○) cholesterol. Note that some of the triglyceride-rich lipoproteins in plasma from the affected mink had been removed by low-speed centrifugation before chromatography (see Materials and Methods). The elution positions of lipoproteins in normal human plasma, run on the same column, are shown by arrows (hVLDL, hLDL and hHDL, respectively).

The great enlargement of the spleen in some animals was probably partly due to accumulation of these cells, but the cellular picture also indicated extramedullar hematopoiesis in this organ. Pulmonary septa were frequently thickened. The arteries were, as a rule, unchanged, although moderate thickening of the arterial walls was occasionally seen in the pancreas, together with vacuolation of medial cells, and, very rarely, accumulation of lipid-laden cells in the intima of arteries in various organs.

Inheritance

Initially animals with hyperlipidemia were obtained from a commercial mink farm in southwest Norway. The number of their normal littermates was, however, not systematically recorded. The Veterinary College then bought animals that had produced offspring with hyperlipidemia from this farm, to raise a small colony in a mink farm owned by and located closer to the Veterinary College. This mink colony has thus far produced 2–5 affected animals annually for 3 years. The heredity of the disease was studied by taking blood samples from all littermates in this colony at weaning at 2 months of age and confirmed by new blood samples taken between 6 months to 2 years later. **Figure 4** shows the family tree obtained. Both sexes were affected. An autosomal recessive mode of inheritance is suggested. The sex of the normal littermates was not systematically recorded.

Plasma lipase activities in normal mink

No previous information was available regarding the lipases involved in lipoprotein metabolism in minks. We therefore studied LPL and hepatic lipase activities in pre- and post-heparin plasma of two normal, male minks in assay systems that were previously much used for human and rat samples. Activities in both lipase assays were low in basal plasma and increased 50- to 220-fold after intravenous injection of heparin. This was similar to what is found in other animal species, but both lipase activities were higher than in humans. The activity in the assay for hepatic lipase was about 600 mU/ml in post-heparin plasma, while comparable values for humans are 338 ± 128 mU/ml (24). Also, in basal plasma, the level of hepatic lipase activity was high, about 10 mU/ml as compared to 0.5–1.5 mU/ml in humans. Assay of LPL was complicated by the fact that we did not have any antiserum that inhibited mink hepatic lipase sufficiently well. Therefore, values from the LPL assay reflect a mixture of hepatic lipase and LPL. This contributes to the very high LPL activities recorded in postheparin plasma, 977 and 1829 mU/ml, compared to 483 ± 180 mU/ml in humans (24).

To avoid problems with specificity of the LPL assay, we separated the lipases by chromatography on columns of heparin-Sepharose and measured the lipase activities in the eluted fractions (**Fig. 5**). Normal minks (Fig. 5A) showed a pattern similar to that in other animal species with two peaks of lipase activity. The first peak, eluting around 0.5 M NaCl, corresponds to hepatic lipase, which was detected to about 75% also in the LPL assay. Then followed a larger peak of LPL activity, which eluted around 0.8 M NaCl. There was approximately twice as much LPL activity as hepatic lipase activity in post-heparin plasma of normal mink. The immunoassay for human LPL detected the active LPL in the second peak, but also a peak of inactive LPL that eluted earlier in the gradient. This pattern is similar for humans (22, 25). The inactive peak presumably represents LPL monomers. The mass ratio of active to inac-

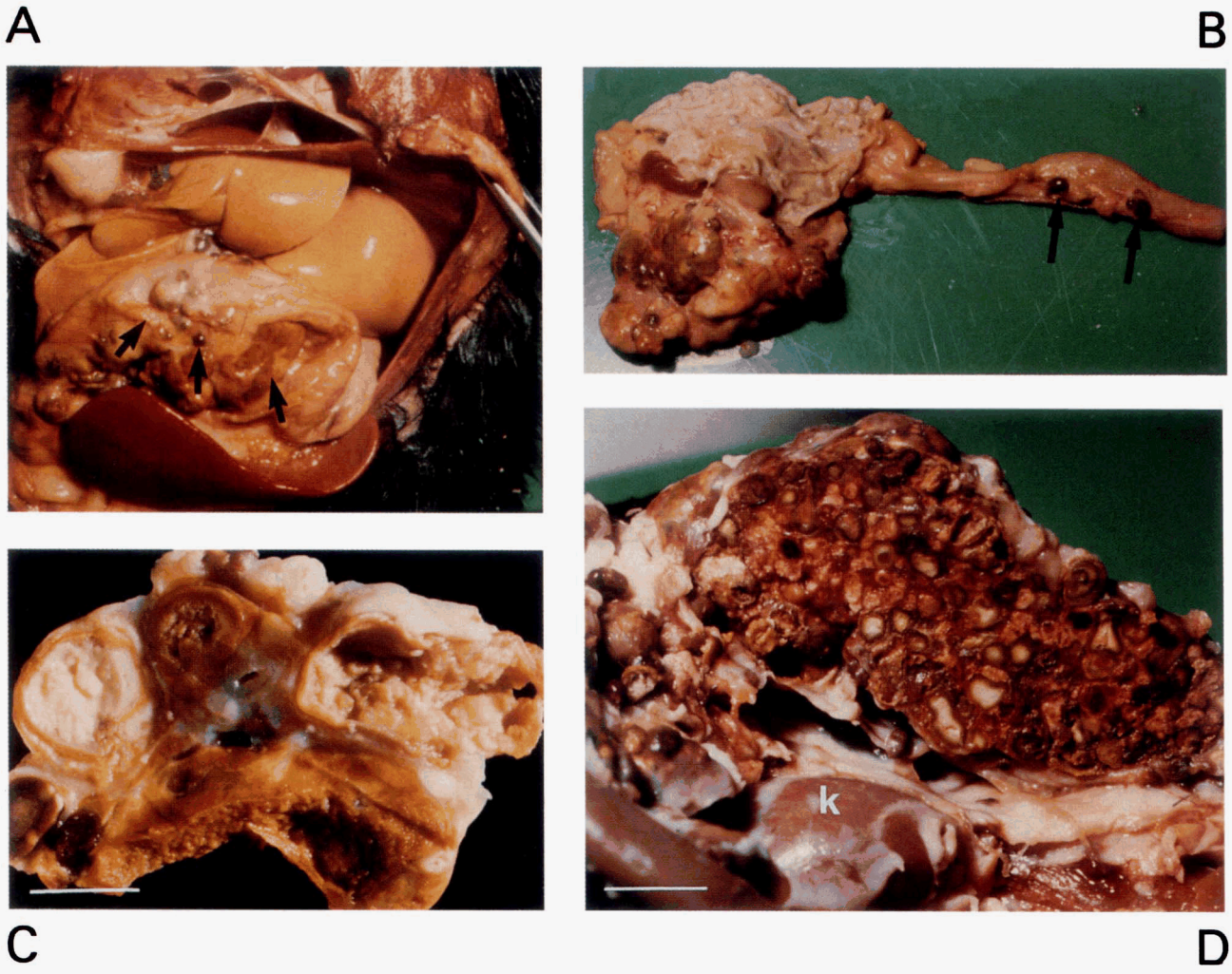

Fig. 2. Macroscopic changes of visceral organs in affected minks. Panels A–C show pictures from a female mink at 10 months of age. Panel D was from another female mink that died at 9 months of age. The arrows point to lipogranulomatous lesions. Panel A: Obvious lipid accumulation and enlargement of the liver and considerable enlargement of the spleen. The arrows point to lipogranulomas in the mesentery. Panel B: The stomach was opened (top left). Lipogranulomas are seen in close association with the stomach. The arrows point to nodular lesions in the pancreas. Panel C: Cut surface of lipogranuloma from mesentery showing areas of coalescing nodules. Brown areas rich in hemosiderin-laden macrophages. Bar = 10 mm. Panel D: Cut surface of lipogranuloma from the mesentery of. Bar = 10 mm, k = kidney.

tive LPL was about 4, which is similar to that in human post-heparin plasma where the ratio is around 3 (25). The specific activity of LPL in the second peak was about 280 U/mg which is comparable to human LPL using the same assay conditions.

Plasma lipase activities in affected minks

Fig. 5B shows separation on heparin-Sepharose of post-heparin plasma from an affected mink. There was only one peak of lipase activity and this had the characteristics of hepatic lipase. The amounts of hepatic lipase activity were similar in the normal and the affected animals. A peak of inactive LPL protein eluted at a slightly lower salt concentration than hepatic lipase, in the same position as the inactive LPL in normal minks. The amount of inactive LPL recovered from the affected animal (574 ng/ml plasma) was similar to that recovered from the normal mink (326 ng/ml plasma).

Lipase mass in basal plasma (pre-heparin) was studied in two other minks. There was 3- to 4-fold more LPL protein in the normal mink than in the affected mink. Most of this corresponded to the early peak of inactive LPL (**Fig. 6A and B**). In the normal mink a low but significant peak of LPL mass eluted in a position expected for active LPL dimers. No significant LPL activity could, however, be detected in the fractions due to the low initial level of activity in the plasma.

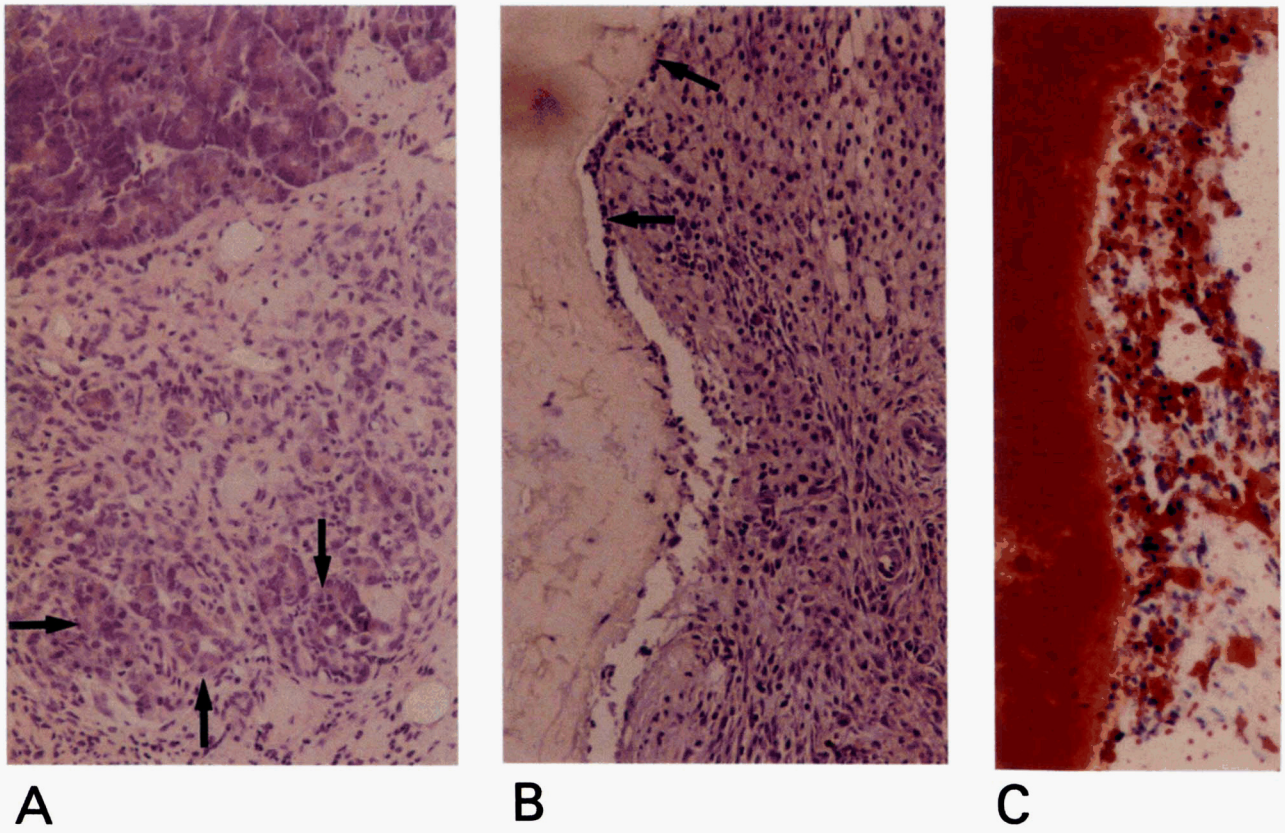

Fig. 3. Microscopic changes in affected minks. All micrographs are from the same animal as in Fig. 2 A–C. Panel A: Lipogranulomatous changes in the pancreas. Unchanged pancreatic tissue is shown in the upper left of the panel. Remnants of pancreatic parenchyma are indicated by arrows; HE stain; magnification × 425. Panel B: Section showing amorphous material occupying spaces in lipogranuloma from mesentery. The arrow points to a flattened cell layer encapsulating the lesion. Foamy macrophages are seen, particularly numerous in the upper right corner; HE stain; magnification × 425. Panel C: Same granuloma as in panel B but this section was Sudan-stained. Sudanophilic substance to the left in the panel corresponds to the amorphic material in panel B. Lipogranulomatous tissue filled with Sudan positive cells is seen to the right.

DISCUSSION

The phenotype of the affected mink, with massive hypertriglyceridemia and an apparent recessive mode of inheritance was highly suggestive of LPL or apoC-II deficiency (1–3). ApoC-II activity was normal (not shown) in the affected minks and post-heparin plasma contained lipase activity. Chromatography on heparin-Sepharose revealed, however, that this was entirely due to a lipase with the characteristics of hepatic lipase. There was no detectable activity in the elution position expected for active LPL. In all species that have been studied this far, including normal mink, active LPL elutes at the high salt position where activity was lacking in the affected mink. Immunoreactive LPL protein was present in plasma of both affected and normal mink, and eluted slightly before hepatic lipase. Such inactive LPL protein has previously been found in plasma of humans (22, 25) and in rat tissues (26). It is not clear whether the inactive protein comes from decay of active LPL or whether it has never folded into the active form. Some patients with LPL deficiency have inactive LPL protein in plasma (3, 27). Expression in eucaryotic cells of LPL with mutations corresponding to those in type I patients have shown that the inactive LPL protein is in some cases retained and degraded within the cells (28) but in other cases it is secreted (2,29). The inactive enzyme proteins present in the expression media behave on heparin-Sepharose like the inactive LPL protein found in plasma of the affected minks (29–31). All in all, the picture in the affected minks is consistent with a point mutation that results in a catalytically inactive LPL that, at least in part, is secreted from cells. Due to the limited number of affected animals available thus far, we have not yet been able to evaluate whether or not the levels of inactive LPL in pre- and post-heparin plasma in the affected mink are significantly different from those in normal mink.

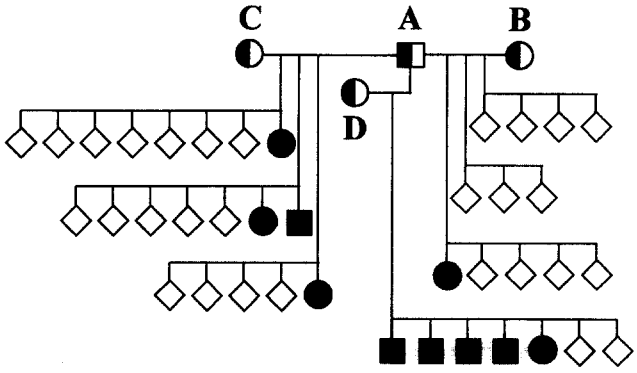

Fig. 4. Family tree from mink colony with hyperlipidemia. The male A (◧) and the females B, C, and D (◐) had previously had offspring with hyperlipidemia but were not hyperlipidemic themselves. A was the brother of B. C and D were his daughters after mating with another female. Not all mink females such as D are successfully impregnated each season. We cannot explain why as many as 5 of her 7 kits were hyperlipidemic at blood sampling at 2 months of age. We cannot exclude the possibility that some kits that may have died in the neonatal period were removed by the keeper and thus not registered; (■, ●) male and female offspring with hyperlipidemia; (◊), offspring without hyperlipidemia.

Normal minks have most of their plasma cholesterol in HDL. The level of LDL-like lipoproteins is low and they appear to contain some triglycerides. It is not known whether minks have cholesteryl ester: triglyceride transfer protein (CETP) activity. Heparin-releasable LPL activity appears to be relatively high as compared to other animal species. Standard mink diet, which is designed to obtain high fur quality at a low price, has a high fat content. In the normal mink, non-fasting triglyceride levels were low, 0.7 ± 0.2 mmol/L, indicating that they are able to metabolize dietary lipids efficiently. The LPL-deficient minks, on the other hand, had severe hyperlipemia. In some of them plasma triglycerides were over 100 mmol/L. When the fat content of the diet was reduced to 16 E%, plasma triglycerides decreased dramatically. This diet still provided relatively much lipid suggesting that the minks have substantial ability to transport triglycerides even without any active LPL. This presumably occurs through clearance of whole chylomicron particles in the liver, possibly with the aid of hepatic lipase, in the reticuloendothelial system and perhaps in other tissues. The mechanisms involved in these clearance processes are presently unknown. Interestingly, although the metabolism of the triglyceride-rich lipoproteins was much disturbed, the levels of LDL and HDL in the affected minks were relatively normal. This is different from the situation in humans where LPL-deficient individuals have low levels of LDL and HDL (3). The increase in plasma cholesterol levels in the affected minks was accounted for by cholesterol in the triglyceride-rich lipoproteins.

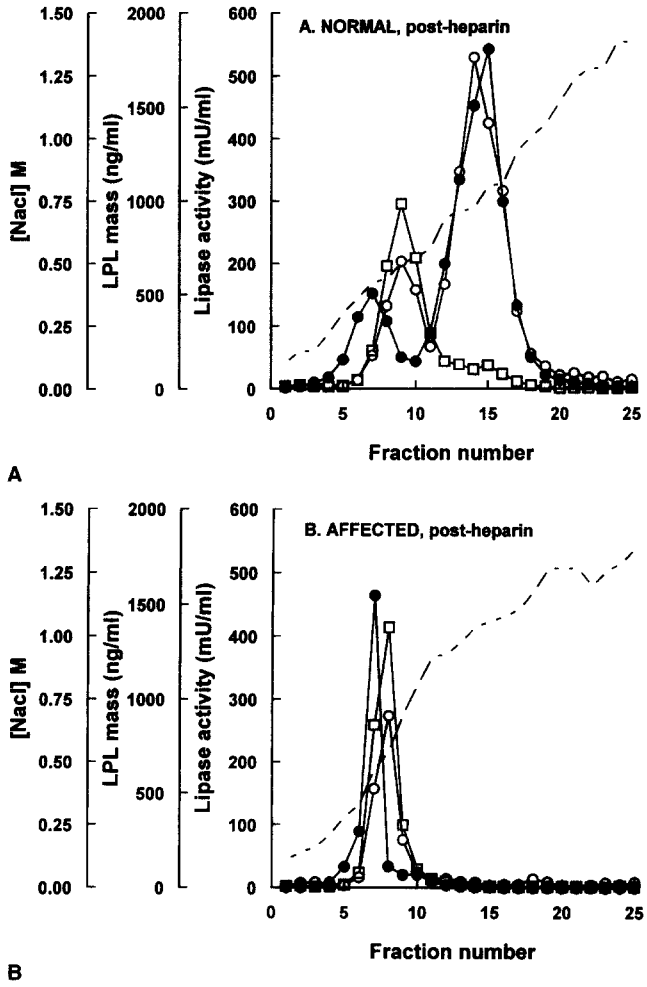

Fig. 5. Separation of lipases in post-heparin plasma by chromatography on heparin-Sepharose. The procedure is described in Materials and Methods. Panel A shows the result of separation of 25 ml post-heparin plasma from a normal, male mink at 5 months of age. Panel B shows the results for 18 ml post-heparin plasma from a male affected mink at 5 months of age (animal no. 2 in Table 1 after 3 weeks on 16 E% fat). (○) Activity in assay for LPL. (□) Activity in assay for hepatic lipase. Note that hepatic lipase is also about 75% active in the LPL assay. (●) Reactivity in immunoassay for lipoprotein lipase (LPL). The dashed lines show the profiles of the salt gradients as determined by conductometry compared to standard solutions made up in the same buffer.

The mechanism by which lipogranulomas form in pancreas and mesentery of LPL-deficient mink is of great interest as the pathogenesis of pancreatitis in hypertriglyceridemic humans is unknown (3). Cats with LPL deficiency also develop lipogranulomatous tissue in the jejunal mesentery, but not in the pancreas (32). A further difference from the minks is that in the cats there are lipogranulomas along the nerve roots and subcutaneous xanthomas (10). Thus, there seem to be underlying factors, as yet unknown, that influence the locations of the granulomas.

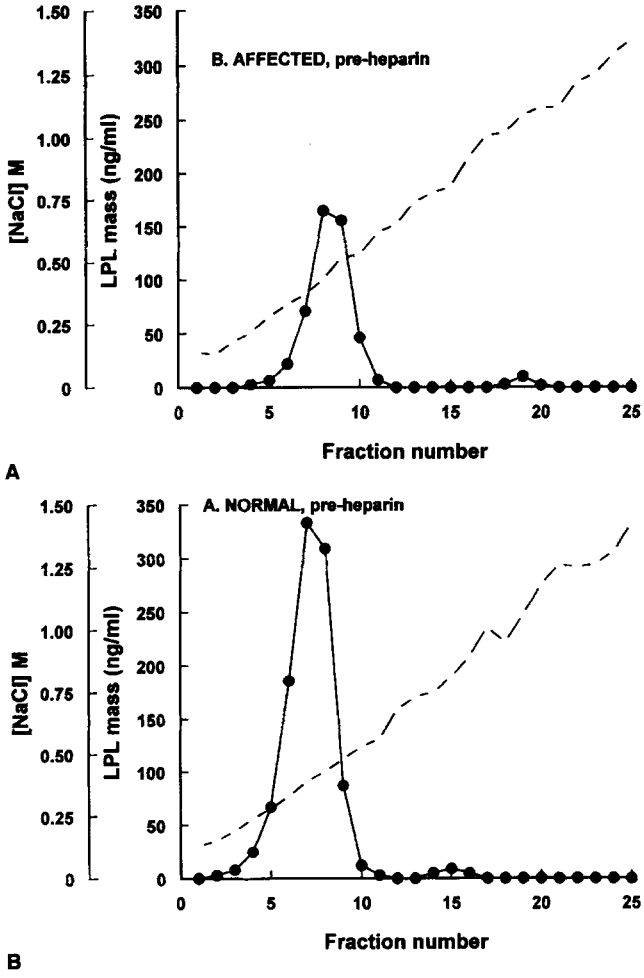

Fig. 6. Separation of lipases in basal plasma (pre-heparin) by chromatography on heparin-Sepharose. Conditions as in Fig. 5, but panel A shows separation of 5.5 ml plasma from a normal mink at 7 months of age and panel B shows separation of 7.5 ml plasma from an affected male mink (animal no. 1 in Table 1 at 7 months of age). Note that these individuals were different from those used in Fig. 5. More than 90% of the LPL mass in plasma bound to the column in both cases.

The lipid in the granulomas may stem directly from lymph chylomicrons but could also come from lipoproteins filtered out from blood. The localization of lipogranulomas in the mesentery suggests that the lipid material comes directly from stagnant lymph. The lesions in the pancreas cannot be explained by such a mechanism as the lymph from the gut does not pass the pancreas. In LPL deficiency, no defect has been described in the formation of chylomicrons in the enterocytes or in the transport of chylomicrons through the lymph vessels of the gut, the mesentery, or the thoracic duct until it is delivered into the venous system.

In humans, the lipoprotein lipase deficiency syndrome has previously been considered to be non-atherogenic. Recently atherosclerosis has been found in middle-aged patients with LPL deficiency (33). There may still be an apparent discrepancy between the hyperchylomicronemia present from childhood and the degree of atherosclerosis. Minks in the present study did not show atherosclerotic changes or other obvious lesions of the arterial walls. The endothelium, intima, and the rest of the coronary artery walls of affected minks were only occasionally very moderately changed. Zilversmit (6,7) has pointed out that a decisive factor may be that the large chylomicron particles do not filter into the vessel walls. It is, however, possible to speculate that the endothelium in some parts of the vascular bed is less impermeable to chylomicrons. If the endothelium in pancreas and mesentery is, to a small extent, permeable to or damaged by such particles, the massive hyperlipoproteinemia in LPL-deficient individuals on a high fat diet might lead to the effusion of nonphysiological amounts of lipoproteins into the surrounding tissue with foam cell formation in these organs. The foam cells may then produce cellular mediators and also disintegrate and thus stimulate the formation of granulomatous tissue.

In conclusion, a new animal model for LPL deficiency has been found. The genetic explanation for the disease, its consequences for lipoprotein metabolism, and early pathological changes will be studied when efficient breeding has been achieved.

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