Hyperlipoproteinemia type I in a patient with active lipoprotein lipase in adipose tissue and indications of defective transport of the enzyme

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Abstract This paper presents a case of typical hyperlipoproteinemia type I in a young woman. Her serum triglycerides varied between 2 and 90 mmol/l and she had substantial amounts of apolipoprotein B-48 in fasting plasma. She had no detectable lipoprotein lipase (LPL) activity in post-heparin plasma (<0.2 percent of normal). Southern blot analysis suggested no major defect in her LPL gene and Northern blot analysis of adipose tissue RNA showed normal-sized LPL-mRNA. A 2-h [^{35}S]methionine incorporation experiment with adipose tissue pieces in vitro showed that she produced normal-sized LPL and had LPL catalytic activity in the tissue. The amounts were, however, only 5–10% of control. No detectable LPL radioactivity or catalytic activity was released from patient tissue even in the presence of heparin in the incubations. Immunofluorescent staining of adipose tissue biopsies from the patient showed LPL immunoreactivity only in adipocytes and little or none within the capillaries. Treatment of immunoprecipitated labeled LPL with endoglycosidase H showed that the oligosaccharide chains on her enzyme were of the high-mannose type and not processed as in controls. Taken together the data suggest that the patient synthesizes a relatively normal LPL protein which is core-glycosylated and folded into active enzyme as in normal subjects, but is not effectively transported via the Golgi to the cell surface. —**Fager, G., H. Semb, S. Enerbäck, T. Olivecrona, L. Jonasson, G. Bengtsson-Olivecrona, G. Camejo, G. Bjursell, and G. Bondjers.** Hyperlipoproteinemia type I in a patient with active lipoprotein lipase in adipose tissue and indications of defective transport of the enzyme. *J. Lipid Res.* 1990. **31:** 1187–1197.

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Decreased lipoprotein lipase (LPL) (EC 3.1.1.3) activity causes severe hypertriglyceridemia (chylomicronemia, hyperlipoproteinemia type I) (1, 2). Primary hyperlipoproteinemia of this kind is rare and associated with a high risk of relapsing hemorrhagic pancreatitis with ultimate pancreatic insufficiency. It may be due either to a primary enzyme defect (review and references in 1 and 2), presence of an LPL inhibitor (3), or to deficient activation of LPL because of lack of (4) or abnormalities in (5) its major activator, apolipoprotein (apo) C-II.

LPL is a dimeric glycoprotein that is synthesized in and secreted from several parenchymal cells including the adipocyte (6–8). Capillary endothelial cells do not themselves synthesize LPL, but bind the active enzyme to their luminal surface (9). LPL has a site that binds to a glycosaminoglycan on the cell surface through specific coulombic interactions (10, 11). This allows the enzyme to get in close contact with substrate lipoproteins. Heparin competes effectively with the binding of LPL to the endothelial cell surface glycosaminoglycans. In normal individuals, but not in subjects with primary LPL deficiency, heparin administration rapidly liberates bound enzyme and increases plasma LPL activity many fold (1, 2).

LPL has another site that binds specifically to apoC-II (12, 13), which is an important activator of LPL (14). Normal hydrolysis of plasma triglycerides by LPL, therefore, requires apoC-II on the surface of substrate chylomicra and VLDL. ApoC-II deficiency is a genetic disorder that leads to defective LPL activity with the clinical course of hyperlipoproteinemia type I (4, 5). Infusion of normal plasma containing apoC-II temporarily restores LPL activity and causes an improvement of the hyperlipoproteinemia.

The synthesis, intracellular processing, and secretion of LPL have been studied in some detail in adipocytes (15–19) and the cDNA encoding LPL has been cloned from five species including man (20–24) and localized to chro-

Abbreviations: LPL, lipoprotein lipase; HL, hepatic lipase; LDL, low density lipoprotein; HDL, high density lipoprotein.

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mosome 8 in both humans (25) and mice (26). The LPL molecule is highly conserved through evolution (10). The regulation of gene expression is largely unknown, but there is evidence of regulation of enzyme mass by intracellular degradation (16, 18). In guinea pigs, feeding increases the expression of LPL-mRNA and LPL activity in adipose tissue fivefold in comparison with fasting (27).

Recent molecular studies have revealed cases of LPL deficiency due to major rearrangements of the LPL gene, such that no LPL protein is made (28, 29) and cases where catalytically inactive LPL is made and secreted (30).

This report presents a case of typical hyperlipoproteinemia type I without significant extracellular LPL activity. It differs from most previously described cases in that the patient synthesizes a normal-sized catalytically active LPL protein in her adipose tissue.

SUBJECTS AND METHODS

The patient

The patient (CK) was a 21-year-old female student at law school. She had neither cutaneous symptoms nor significant history of other disease. Her gynecological history was normal and she was not taking oral contraceptive pills. Her parents and younger sister were healthy. Her father had about 3 mmol/l serum triglycerides. He was of Polish-Jewish ancestry and had lost contact with all relatives during World War II. The maternal relatives were Swedish. No cases of hyperlipoproteinemia or pancreatitis were known among the relatives.

At 15 years of age, CK was subjected to appendectomy. Lipemic serum was noticed and serum triglyceride and cholesterol levels ranged 15–23 and 5.6–7.2 mmol/l, respectively. Lipoprotein electrophoresis showed a grossly increased chylomicron fraction, some increase in the prebeta and some decrease in the alpha lipoprotein bands. Type I hyperlipoproteinemia due to insufficient LPL activity or apoC-II deficiency was considered and CK was put on a low-fat (40 g/d) diet.

Since 16 years of age, she suffered nine attacks of pancreatitis as diagnosed by clinical symptoms and signs as well as serum amylase levels. All attacks coincided with occasional increases in serum triglyceride levels. Although she needed morphine, none of her attacks was clinically severe or hemorrhagic and her symptoms always disappeared within 1 or 2 days of fasting.

Other routine laboratory tests were normal. A moderate enlargement of the spleen has been observed since CK was 15 years of age. No abnormalities in the liver or pancreas were found by ultrasound investigation.

The patient agreed to undergo one diagnostic surgical biopsy of subcutaneous abdominal fat. This was undertaken during general anesthesia and the specimen was divided for morphology, biochemical experiments, and RNA preparation.

This study was approved by the local ethical committee and performed after informed consent from CK.

Control subjects

Sera for control experiments were obtained from healthy normolipidemic subjects. Subcutaneous abdominal adipose tissue for control experiments was obtained from a normolipoproteinemic middle-aged woman undergoing laparotomy due to suspect perforation of an ulcer of the stomach. Pre-operatively, she fasted overnight and she was operated under general anesthesia. A slow glucose (5.5 percent w/v) infusion was started at induction of anesthesia. No signs of disease were, however, found during laparotomy or afterwards.

Clinical laboratory procedures

Serum cholesterol and triglyceride as well as HDL-cholesterol levels were determined by enzymatic procedures as previously described (31). Apolipoproteins A-I (apoA-I) (32), B (apoB) (31), and E (apoE) (33) were determined by electroimmunoassay and apoC-II by radioimmunoassay (34), as previously described. Other clinical laboratory determinations were performed according to routine procedures.

Subfractionation of chylomicrons and VLDL was carried out by cumulative rate centrifugation to float particles of diameter 75 nm (S_f 400), diameter 50–75 nm (S_f 175–400), diameter 37–50 nm (S_f 100–175), and 20–37 nm (S_f 20–100), as suggested by Lindgren, Jensen, and Hatch (35) and described in detail by Redgrave and Carlson (36). LDL was obtained as a well-defined band after the final centrifugation and was recovered by aspiration. The infranate after the final centrifugation was adjusted to a solution density of 1.21 g/ml, centrifuged for 40 h at 50,000 rpm in a Beckman 50 rotor, and HDL was recovered in the supernate.

The lipoprotein fractions, adjusted to approximately 1 mg/ml of protein, were delipidated by adding 0.5 ml to 5 ml of methanol–chloroform 60:40 (v/v) at – 20°C. The mixture formed a single phase and was left to stand overnight at – 20°C. The precipitated apolipoproteins were collected by centrifugation at 1000 *g*, the supernatant was decanted, and the remaining solvent was evaporated under a gentle N_2 stream without completely drying the pellet. The precipitated proteins were dissolved in 0.5 ml of sample buffer made of 50 mM Tris-HCl (pH 6.8), 2% mercaptoethanol, 4% sodium dodecyl sulfate (SDS), 12% glycerol, and 0.01% Coomassie Brilliant blue. The samples were heated for 10 min at 65°C before application to the gel. The rest of the electrophoretic procedure followed the Tricine-SDS system described by Schägger and von Jagow (37), except that an LKB 2001 (LKB, Bromma, Sweden) cell was used. The gels were 1.5 mm

thick and made of a 5% acrylamide stacking segment and a 10% running gel. For the electrophoretic separation, 150 V (constant voltage) was applied for 6 h. The gels were fixed and stained with Coomassie Brilliant blue. The molecular weight standards and reagents were obtained from Bio-Rad (Richmond, CA).

Biochemical procedures

Lipase activities were determined in plasma obtained immediately before and 30 min after 5000 IU heparin i.v. as previously described (38) with and without inhibition of hepatic lipase (HL) by a specific antiserum (39).

To test whether CK's plasma might contain an inhibitor for LPL, purified bovine LPL (40 mU/ml) was added and the sample was incubated at 37°C for 40 min. In a simultaneous control experiment, Intralipid (KabiVitrum, Stockholm, Sweden) was added to a sample of normal plasma to give the same triglyceride concentration as in CK's plasma. The production of free fatty acids was measured by titration.

Synthesis and secretion of LPL in adipose tissue was studied in vitro, as previously described (16). Briefly, pieces of adipose tissue obtained by a surgical biopsy were incubated in the presence of [^{35}S]methionine in medium with heparin (33 μg/ml) or without heparin for 2 h. Aliquots of media and of tissue homogenates were immunoprecipitated with a rabbit antiserum against bovine LPL. This antiserum was known to cross-react with human LPL. The washed immunoprecipitates were redissolved and run at the same amounts of total protein radioactivity from CK and control samples on SDS polyacrylamide gels. The LPL band was identified, cut out, and counted as previously described (16). The glycosylation patterns of LPL immunoprecipitated from tissue homogenates were studied by treatment with endo-β-N-acetylglucosaminidase H (endo H) (40 mU/ml) and subsequent SDS polyacrylamide gel electrophoresis (16). In this case, the bands were quantitated by densitometric scanning of autoradiographs.

LPL activities in adipose tissue were studied in the same experiment as [^{35}S]methionine incorporation. The procedure has been described elsewhere (16).

The expression of mRNA for LPL in adipose tissue was studied on total RNA prepared by the method of Chirgwin et al. (40). Electrophoresis in a 1% agarose gel in 40 mM 3-[N-morpholino] propanesulfonic acid, pH 7.0, was carried out after denaturation with glyoxal and dimethylsufoxide (41). The same amounts of total RNA from CK and the control subject were applied to the gel. The glyoxylated RNA was then transferred onto nitrocellulose filters (42). The blots were then hybridized with a human LPL-cDNA probe (provided by Dr. Richard Lawn, Genentech Inc.) which spanned from nucleotides 1 to 2413, as presented by Wion et al. (23). The probe was labeled with [alpha-^{32}P]-dCTP by the oligolabeling technique (43).

Prehybridization and hybridization were carried out under stringent conditions with 50% formamide at 46°C (44). Posthybridization washings were also performed at high stringency (0.1% SDS and 0.1 × SSC [0.15 M NaCl and 0.015 M sodium citrate, pH 7.6] at 64°C). LPL-mRNA was quantitated by densitometric scanning.

Studies on the LPL gene

Blood for genomic studies was collected in EDTA Vacutainer Tubes (Becton Dickinson, Meylan Cedex, France). DNA was prepared from blood as described by Blinn and Stafford (45). Genomic mapping was carried out after cleavage with restriction enzymes (BamHI, Sph I, Stu I, and Xba I) and hybridization with the human LPL-cDNA probe as described (23).

Immunohistochemistry

An evaluation of the distribution of LPL in an adipose tissue specimen was performed utilizing indirect immunofluorescence with a specific antiserum against LPL (primary antibody) and a fluorescein (FITC)-labeled antiserum (secondary antibody) as previously described (46). The observations were compared with those of healthy volunteers among the laboratory staff.

RESULTS

Clinical observations

The concentrations of serum lipids and apolipoproteins are given together with reference values in **Table 1**. Over the observation period of 6 years, CK's serum triglycerides varied widely (median 21.3, range 1.7–87 mmol/l) (**Fig. 1**). The nine attacks of acute pancreatitis occurred at serum triglyceride levels above 20 mmol/l, although attacks did not always occur above this level (Fig. 1). The lowest levels were observed after fasting for a few days under controlled circumstances in the hospital. Many

TABLE 1. Serum lipid and apolipoprotein levels in comparison with controls

Fraction	CK Median	CK (Range)	Reference Mean ± SD
Triglycerides (mmol/l)	21.3	(1.7–87)	0.97 ± 0.45[a]
Cholesterol (mmol/l)	7.1	(3.9–18)	5.99 ± 1.13[a]
HDL cholesterol (mmol/l)	0.41	(0.24–0.69)	1.65 ± 0.40[a]
ApoA-I (g/l)	0.79	(0.69–1.18)	2.24 ± 0.37[a]
ApoB (g/l)	1.04	(0.47–1.43)	1.16 ± 0.29[a]
ApoC-II (mg/l)	57	(54–59)	23 ± 9[b]
ApoE (% of standard)	40	(25–55)	94 ± 18[a]

[a]Johansson et al. (33).
[b]Gustafson et al. (34).

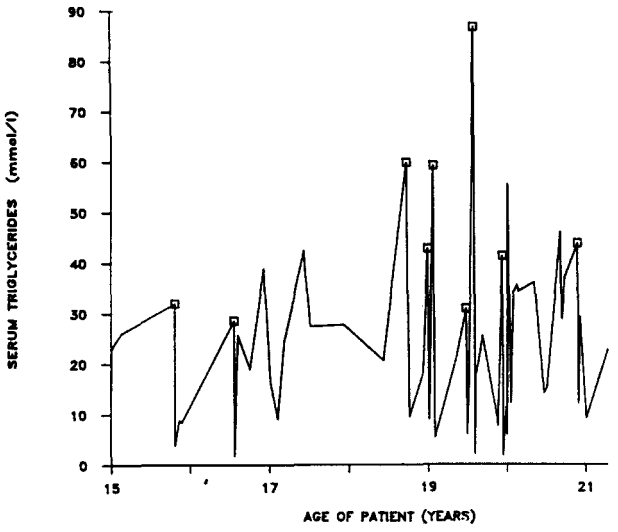

Fig. 1. Variations in serum triglyceride levels during 6 years of follow-up of the patient CK. Boxes indicate attacks of acute pancreatitis.

high values were observed after occasional neglect of dietary recommendations.

The variations in serum cholesterol levels were also great (median 7.1, range 3.9–18 mmol/l), but less pronounced. The correlation with serum triglycerides was very strong ($r = 0.94$, $P < 0.01$). Serum apoB levels neither correlated with serum triglyceride nor with serum cholesterol levels and varied less. HDL-cholesterol varied inversely with serum triglycerides ($r = -0.36$, $P = 0.027$). HDL cholesterol as well as serum levels of apoA-I were much lower than in a random population sample of 40–55-year-old women (32). CK's serum levels of apoE were also lower than in this control sample. CK's apoC-II levels were about twice that of normals (34). In vitro incubations showed that CK's plasma activated a labeled triglyceride emulsion for hydrolysis by bovine LPL (data not shown). Thus, her apoC-II was functional.

Lipoprotein electrophoresis showed faint bands corresponding to alpha and beta lipoproteins. There was a strong band corresponding to chylomicrons and a considerable degree of tailing into the beta and prebeta positions. Cumulative rate ultracentrifugation yielded low HDL and LDL cholesterol and triglycerides, respectively (**Table 2**). More than 90% of the serum triglycerides floated at d 1.006 g/ml and a gradual decrease in triglyceride content from fractions S_f 400 to S_f 20–100 was found. After subfractionation of chylomicrons, VLDL and intermediate fractions, we observed a dominance of B-48 in a pure chylomicron fraction and of B-100 in the fraction of small VLDL particles (**Fig. 2**). Intermediate fractions contained both B-48 and B-100. In addition, apoE was present both in chylomicrons and in the VLDL fractions. C peptides were observed even when the resolution in that range of molecular sizes was poor in the present system. The peptide distribution in LDL and HDL was not different from that normally observed.

During the first attack of pancreatitis, CK was given an infusion of heparin (100 IU/h) after a bolus dose of 500 IU. The infusion was interrupted for a period between 8 and 12 h after onset during which she obtained two infusions of 100 ml normal plasma each. Plasma infusion had no significant effect on her triglyceride levels (data not shown). We could not, however, determine whether heparin, fasting, or both caused the decrease in serum triglycerides from 32 to 4 mmol/l that was observed during the first 30 h.

Consequently, CK was readmitted during a symptom-free period and subjected to a controlled dietary treatment (**Fig. 3**). During the first 7 days, serum triglycerides fell from 20 to a plateau about 14 mmol/l on a 40 g/d fat diet. A further decrease to a new plateau at 7 mmol/l was obtained when dietary fat was reduced to 20 g/d. During this period, CK obtained heparin 5,000 IU as single i.v. injections on three separate occasions (Fig. 3). Nothing suggests that this had any effect on serum triglyceride levels. Therefore, the conclusion was that LPL activity in plasma was not induced by heparin administration.

During a severe attack of pancreatitis, plasmapheresis of 2.5 liters of turbid plasma was undertaken and withdrawn plasma was substituted with the same volume of normal blood bank plasma. In 2 days, the serum triglycerides fell from 87 to 3 mmol/l and the patient became free of symptoms.

When purified LPL was added to CK's plasma in vitro, free fatty acids were generated to a similar extent as in a simultaneous control experiment (not shown). This seemed to exclude the presence of an LPL inhibitor in CK's plasma.

Biochemical observations

Injection of heparin released no detectable LPL activity into CK's plasma, but did release HL activity as determined in vitro (**Table 3**). LPL was determined after inhibition of HL by a specific antiserum. If the inhibition of HL was not absolute, residual HL activity rather than LPL activity may account for the very low lipase activity observed in CK's post-heparin plasma. The values corresponded to less than 0.5% of the activity in post-

TABLE 2. Triglyceride and cholesterol levels in different density fractions obtained by cumulative rate ultracentrifugation

Fraction	Triglycerides	Cholesterol
	mmol/l	
Serum	10.7	4.0
Supernatant, d 1.006 g/ml		
S_f 400	3.91	0.86
S_f 175–400	2.83	0.76
S_f 100–175	1.70	0.21
S_f 20–100	0.70	0.35
LDL	0.18	0.55
HDL	0.14	0.23

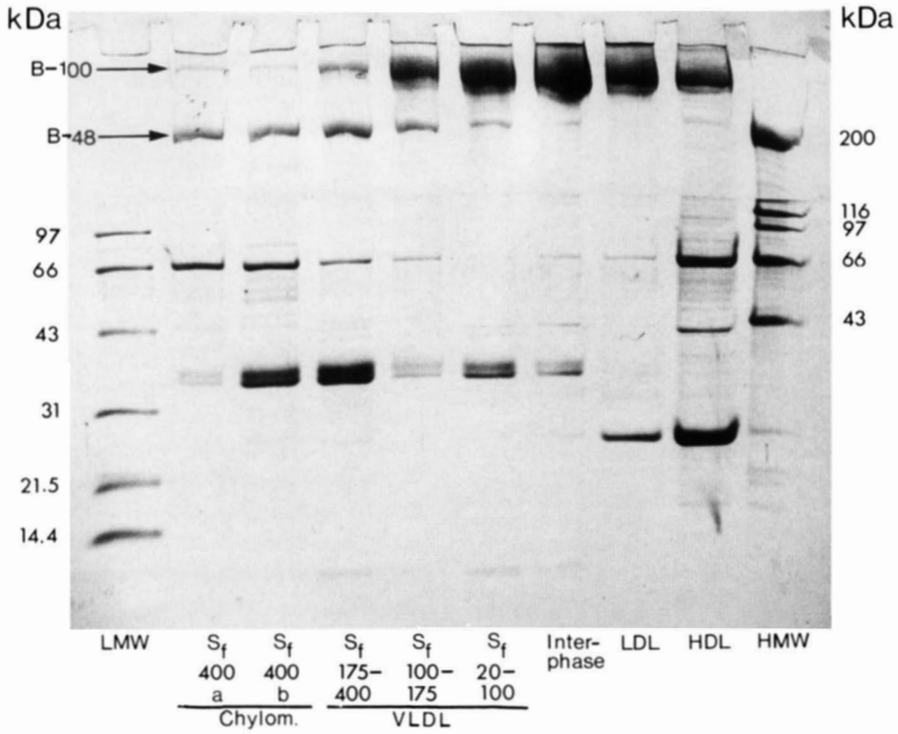

Fig. 2. SDS polyacrylamide gel electrophoresis of delipidated proteins from chylomicrons, different VLDL fractions, LDL and HDL from the patient. Low (LMW) and high (HMW) SDS-PAGE molecular weight standards (Bio-Rad, Richmond, CA) are included. The original S_f 400 fraction (a) was concentrated by recentrifugation (b).

heparin plasma from normal individuals. In fact, the registered activity in CK's post-heparin plasma was less than the LPL activity in pre-heparin plasma of normal individuals (Table 3). The activity of HL (73 mU/ml) was also somewhat low. We did not have comparable normal material, but values in a group of young men in our laboratory were 299 ± 27 mU/ml. According to the literature (47), values in young females are about half of those in males. It is possible, however, that the large amounts of triglycerides in CK's plasma interfered with the assay to give false low values.

To study the synthesis of LPL protein, pieces of adipose tissue from CK and a control subject were incubated with [^{35}S]methionine for 2 h. Tissue homogenates and media were immunoprecipitated and displayed on SDS gels (**Fig. 4**). The results showed the presence of a band in precipitates from the tissue fraction at the position expected for LPL (lanes 4 and 5.). The fraction of total protein radioactivity constituting immunoprecipitable LPL was much less in CK than in the control subject (**Table 4**). During incubation of adipose tissue pieces from the control subject, substantial amounts of immunoprecipitable labeled LPL appeared in the medium. This release was greatly augmented by the addition of heparin to the medium (lanes 7 and 8). In contrast, adipose tissue from CK did not release detectable amounts of LPL to the medium (lanes 9 and 10) (Table 4).

The intracellular transport and processing of LPL was studied by subjecting immunoprecipitated enzyme from adipose tissue homogenates to treatment with endo H and subsequent gel electrophoresis. In the control sample (**Fig. 5B**), the slower moving component presumably represents a form of the enzyme where one or more oligosaccharide chains had been processed and, hence, made resistent to endo-H. In the patient, the faster moving band dominated (Fig. 5D). Compared to the control, where the slower moving (processed) molecules dominated, the results for CK suggest a transport defect.

In the same experiments, LPL activity was measured in the adipose tissue pieces and in the medium (**Table 5**).

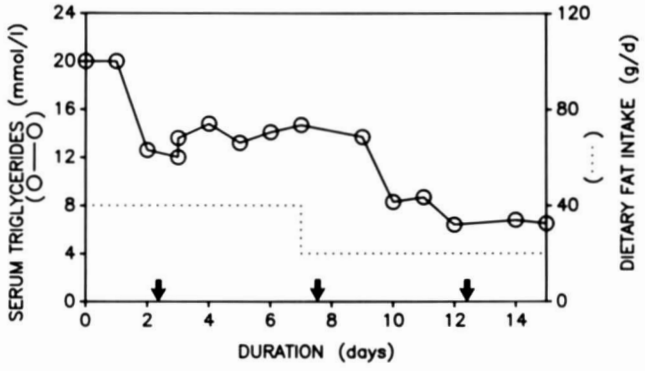

Fig. 3. Effect of dietary fat and heparin infusion on serum triglyceride levels (O) of the patient CK. A fat intake of 40 and 20 g/day (dotted line) as well as intermittent i.v. injections of heparin 5000 IU (arrows) were tested in the hospital over a period of 15 days during a symptom-free interval.

TABLE 3. Lipase activities in pre- and post-heparin plasma from patient CK and normolipoproteinemic control subjects

Plasma	Hepatic Lipase Activity	Lipoprotein Lipase Activity
	mU/ml (mean ± SD)	
Pre-heparin		
Normal[a]	0.40 ± 0.05	1.10 ± 0.13
CK[b]	0.03 ± 0.01	0.25 ± 0.14[c]
Post-heparin		
Normal[d]	299 ± 27	342 ± 30
CK[b]	73 ± 10	0.55 ± 0.14[c]

[a]Samples from seven normal subjects measured together with the CK samples.

[b]Means from assay of three different volumes of serum, each assayed in triplicate.

[c]This analysis was repeated on pre- and post-heparin plasma obtained at another occasion. Post-heparin plasma values were 0.62 ± 0.26 mU/ml, whereas pre-heparin plasma values did not differ from the blanks.

[d]Samples from seven normal young males 10 min after 100 IU heparin/kg body weight.

Large amounts of unincorporated [^{35}S]methionine were present in the media and, although efficiently removed during extraction of the released fatty acids, contributed a relatively high background value in the measurement of LPL activities in the media. The results showed clearly that adipose tissue pieces from CK had substantial LPL activity, 10% (without heparin) or more (with heparin) of the activity found in the control adipose tissue. In the presence of heparin, adipose tissue from the control but not from CK released (detectable) LPL activity.

Genetic observations

The Northern blot hybridizations of total RNA showed that CK had LPL-mRNA of normal size in her adipose tissue, but that the amount was about half that of the control (**Fig. 6**).

Genomic mapping after cleavage with the restriction enzymes Bam HI, Sph I, Stu I, and Xba I and hybridization with the human LPL-cDNA probe showed no major differences from a normal control subject (data not shown).

Morphological observations

Immunofluorescence for LPL was considerably lower in the adipose tissue of the patient than in healthy controls. This is evident from **Fig. 7** in which the two photomicrographs represent the same time of exposure. In addition, the distribution of immunoreactivity was quite different in the patient. Thus, the specific (FITC) fluorescence was found in adipocytes, but not in pericytes or capillaries. This was evident from the faint brick-red (unspecific) autofluorescence that dominated completely over the specific (FITC) fluorescence in CK's capillaries.

DISCUSSION

Clinically, patient CK had all the characteristics of type I hyperlipoproteinemia with an early onset of attacks of pancreatitis. In response to dietary fat intake, her serum triglyceride levels varied enormously and, strongly correlated with this, also her serum cholesterol levels.

More than 90% of the triglycerides was recovered in lipoproteins of $d < 1.006$ g/ml at ultracentrifugation. Although we lack results of cumulative rate ultracentrifugation from an acceptable control population, CK's results diverged grossly from those anticipated for normals and were used primarily for qualitative conclusions. There was a gradual decrease in triglyceride content from S_f 400 through S_f 20. All these fractions contained apoB-48 by qualitative gel electrophoresis. This suggested that they represented mainly intestinal lipoproteins in varying stages of degradation. Qualitatively, apoB-100 was also noticed among the smaller particles, which suggested that

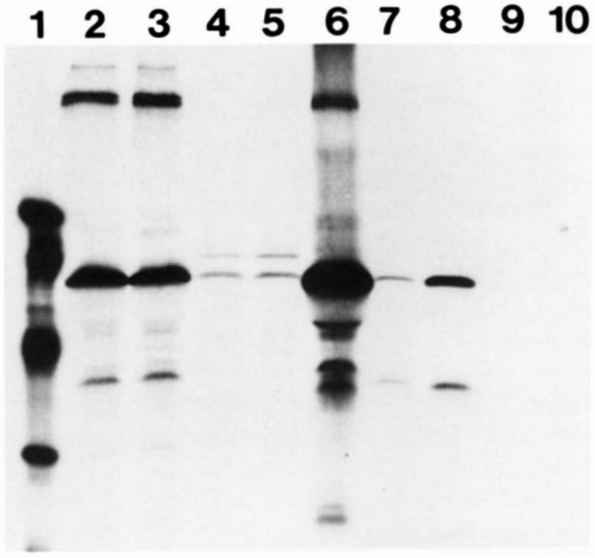

Fig. 4. Synthesis and secretion of LPL in an adipose tissue biopsy from the patient CK and a control subject. Pieces of the adipose tissue were incubated for 2 h in medium with [^{35}S]methionine with (33 μg/ml) and without heparin, as detailed in Biochemical Procedures. LPL was immunoprecipitated from homogenates of the tissue pieces and from the media. For this, aliquots from the tissue homogenates from both CK and the control contained the same protein radioactivity. Samples were analyzed by SDS polyacrylamide (12.5%) gel electrophoresis. The figure shows a fluorogram of the gel. Lane 1: molecular weight standards were phosphorylase B (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (42.7 kDa), and carbonic anhydrase (31 kDa); lanes 2 and 3: immunoprecipitates from normal tissue (lane 2 without and lane 3 with heparin); lanes 4 and 5: immunoprecipitates from CK's tissue (lane 4 without and lane 5 with heparin). A control with nonimmune serum showed that the upper of the two main bands was nonspecific (not shown); lane 6: immunoprecipitate of ^{35}S-labeled LPL from guinea pig adipose tissue; lanes 7 and 8: immunoprecipitates from control medium (lane 7 without and lane 8 with heparin); lanes 9 and 10: immunoprecipitates from CK medium (lane 9 without and lane 10 with heparin)

TABLE 4. Quantitation in tissue pieces and media of immunoprecipitable ^{35}S-labeled LPL produced by incubation of adipose tissue from the patient (CK) and a normolipoproteinemic control subject in vitro

		Tissue (per g wet weight)			Media
Subject	Heparin	^{35}S in Total Protein	^{35}S in LPL	LPL	^{35}S in LPL
		cpm × 10^{-6}	*cpm* × 10^{-3}	*% of total*	*cpm*
Control	–	52.4	27.2	0.052	122
Control	+	65.7	33.5	0.051	594
CK	–	40.3	0.93	0.0023	ND
CK	+	48.2	1.11	0.0023	ND

Data from experiment in Fig. 5; ND, not detectable.

these fractions contained hepatic lipoproteins to some extent. Quantitatively, apoB levels were, however, neither increased nor correlated with serum triglyceride or cholesterol levels and they varied much less. ApoE levels were low and their variation was of a smaller magnitude. LDL as well as HDL cholesterol levels were low and there was a negative correlation between HDL cholesterol and serum triglyceride levels. Combined, the results were compatible with hypertriglyceridemia due primarily to severe chylomicronemia. The presence of chylomicronemia even after a prolonged fasting period suggested a compromised process of breakdown of chylomicrons.

Our attempts to prevent attacks of pancreatitis were not successful. The only way to reduce and control the patient's chylomicronemia was by dietary means. Serum triglyceride levels were largely correlated with daily fat intake. Fasting was most effective in reducing serum triglyceride levels although it was less effective than in normal subjects.

Administration of normal plasma did not influence serum triglyceride levels in the patient and her serum contained functional apoC-II. Thus, apoC-II deficiency did not explain the disorder in our patient.

Addition of purified bovine LPL to the patient's plasma in vitro resulted in a rapid hydrolysis of her lipoprotein triglycerides, which demonstrated that CK's plasma did not contain any LPL inhibitor. These observations suggested that she had a primary LPL defect.

Observations in vivo, ex vivo, and in vitro consistently suggested that CK had a defect in the intracellular transport and secretion of LPL. Moreover, Northern blot analyses showed an amount of normal-sized LPL-mRNA in adipose tissue that was 50% of normal.

In vivo, heparin administration failed to improve her hypertriglyceridemia. Furthermore, heparin did not induce a substantial release of LPL activity into the plasma of the patient. Normal subjects increase their LPL activity in plasma after heparin administration about 200-fold (G. Bengtsson-Olivecrona, unpublished observations).

The ex vivo LPL activity after heparin was far below (1/500) that expected in normal post-heparin plasma.

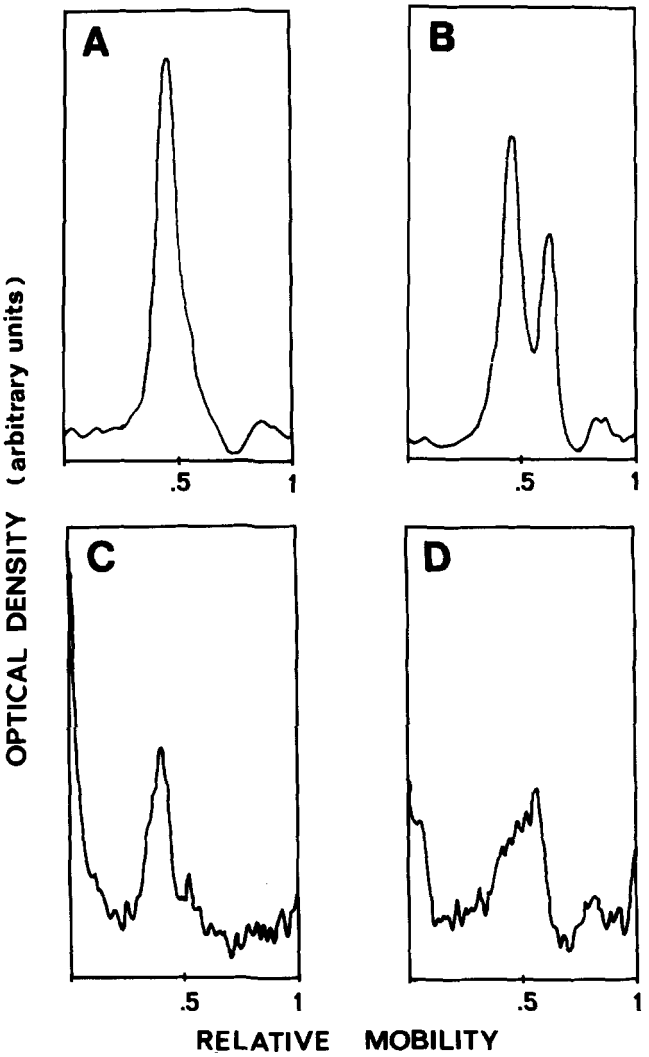

Fig. 5. Analyses of glycosylation patterns of ^{35}S-labeled LPL (c.f. Fig. 6 and Tables 4 and 5) by treatment with endo H. The samples were analyzed by SDS polyacrylamide (10%) gel electrophoresis. The bands corresponding to LPL were quantitated by densitometric scanning. The abscissae give the relative electrophoretic mobility and have been aligned to correspond to the start and front of the electrophoresis. The ordinate gives the absorbance in arbitrary units. Upper panel shows LPL derived from control tissue without (A) and with (B) subsequent treatment with endo H. The lower panel shows the same for CK's tissue without (C) and with (D) subsequent treatment with endo H.

TABLE 5. LPL activities in adipose tissue pieces and in media obtained by incubation of tissue from the patient (CK) and a normolipoproteinemic control subject

Subject	Heparin	LPL Activity	
		Tissue	Media
		mU/mg DNA	
Control	–	421	NS
Control	+	293	22
CK	–	44	NS
CK	+	38	NS

Same experiment as in Fig. 5 and Table 4; NS, not significantly above background.

Immunofluorescent staining with LPL antiserum demonstrated the presence of immunoreactive LPL in adipocytes of the patient, but there was no specific immunofluorescence from LPL within capillaries. In the controls, immunofluorescence from LPL was mainly localized within capillaries. Thus, the results in CK were consistent with a defective secretion.

Cleavage with several restriction enzymes showed no major deletions or aberrations in the coding sequence of the patient's LPL-DNA. This suggests that CK differs from other cases in the literature (28, 29). It does not, however, exclude the possibility of minor changes. Point mutations in the LPL gene leading to defective LPL activity have indeed been described (30). In our case, however, the activity of LPL seemed to be within the normal range although the production of enzyme was low.

Northern blot hybridization of adipose tissue RNA with an LPL-cDNA probe showed the presence of normal-sized LPL-mRNA. The amount was, however, only half of that of our control. Although the results in this study do not allow a firm conclusion, a half-normal amount of normal-sized mRNA would be the expected finding in a heterozygote in which one allele is not transcribed. Alone, one defectively transcribed LPL allele would hardly lead to the severe disorder of our patient. Combined with the transport defect suggested by the in vitro experiments it may, however, lead to severe LPL deficiency, although none of the parents expressed such a defect phenotypically.

The father and mother of our patient were unrelated with Polish-Jewish and Swedish ancestry, respectively. They were healthy, and neither had known heredity for lipoprotein disorders. The father's serum triglycerides were slightly elevated. Therefore, it cannot be excluded that he is a heterozygote for LPL deficiency (48). Our patient may, thus, be a compound heterozygote carrying one allele that does not produce any LPL-mRNA and another that results in synthesis of the catalytically active but defectively transported form of LPL demonstrated here.

In vitro experiments gave further support for a defective production and secretion of LPL in the patient. In the [^{35}S]methionine experiment, much less labeled LPL was recovered from CK than from control tissue, only about 5%. In this experiment, the adipose tissue pieces were incubated for 2 h. Experiments in several laboratories have shown that LPL is released from adipocytes soon after synthesis, or degraded. Hence, the amount of labeled LPL isolated at 2 h does not allow an estimate of the rate of synthesis. It is, however, likely that LPL synthesis was about half normal in CK's adipose tissue as suggested by the mRNA level and that most of the enzyme was rapidly degraded.

The biochemical studies suggested that LPL was not transported normally in the patient. Her adipose tissue had about 10% of control LPL activity. If a commensurate amount of LPL had been transported to the vascular endothelium, it should have been easily detected in post-heparin plasma. There was, however, no detectable LPL activity in the patient's post-heparin plasma. The immunohistochemical studies also indicated that LPL was not transported out of the adipocytes to nearby capillaries. There was a faint but positive immunofluorescence over the adipocytes, but little or no reaction associated with capillaries or other blood vessels. The results were in full accord with the biochemical demonstration of a low amount of LPL within but very little secretion from the adipocytes. In the control subject, most of the LPL after a 2-h labeling period was resistant to endo-H, indicating that the enzyme had reached or passed the Golgi where oligosaccharide chains had been processed and, hence, rendered resistant to endo-H digestion. This is in accord with results from studies with adipocytes from mice (49), guinea pigs (17), and rats (50). In contrast, most of the patient's LPL was sensitive to endo-H, suggesting that it had not been transferred from the endoplasmic reticulum to the Golgi. Lehrman et al. (51) have described a similar defect in the LDL-receptor. They found a point mutation in the gene for the receptor leading to defective glycosylation of a kind similar to the present. They also found premature termination of transcription and severe impairment of LDL receptor function.

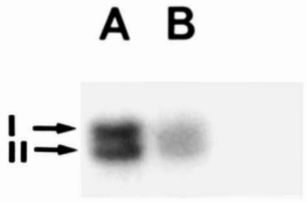

Fig. 6. Northern blot hybridizations of 25 μg total RNA derived from adipose tissue of (A) a normolipidemic control subject and (B) patient CK. Roman numerals I and II indicate mRNA sizes of 3.7 and 3.3 kb, respectively.

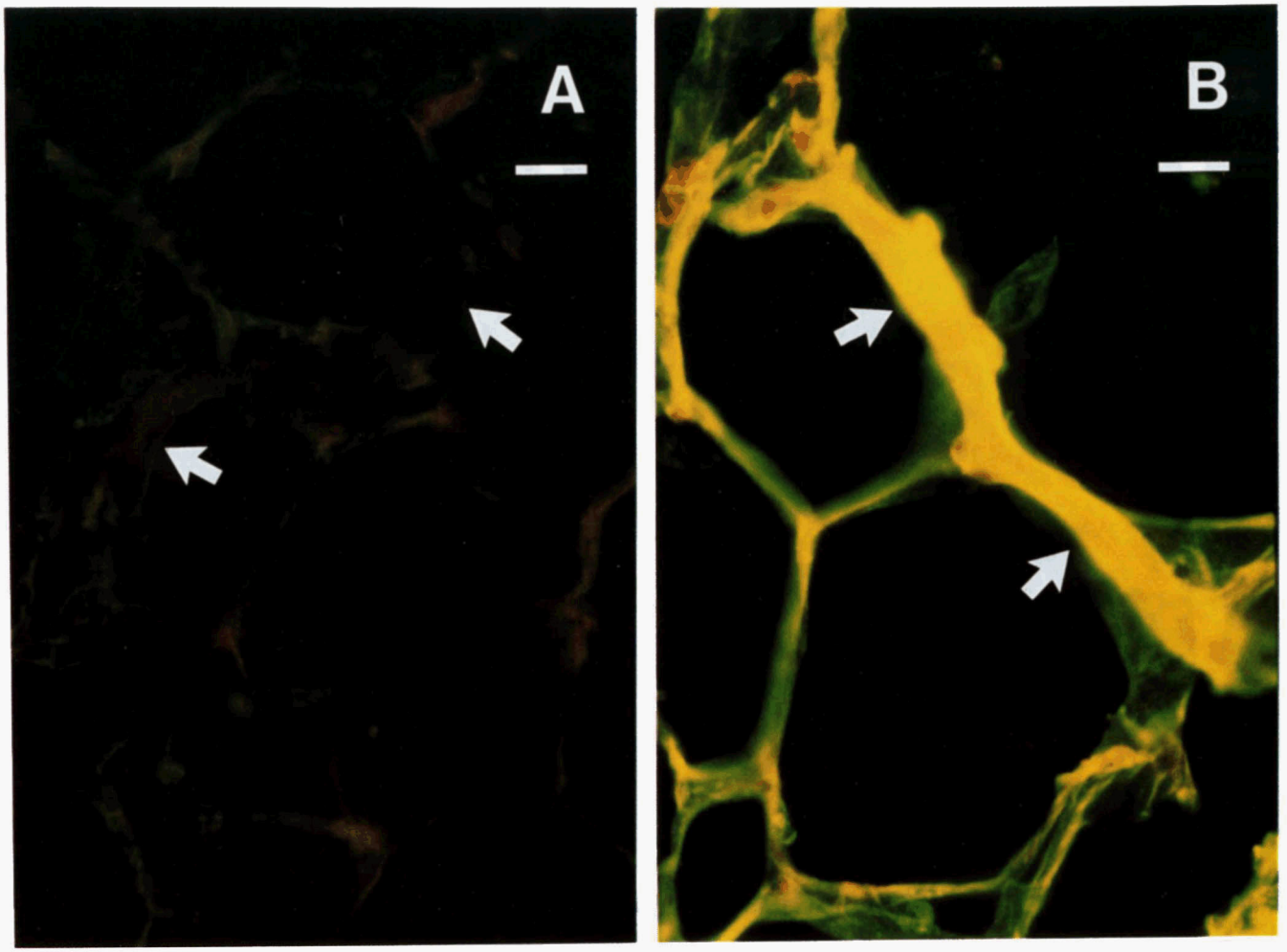

Fig. 7. Indirect FITC immunofluorescence showing the distribution of LPL in adipose tissue biopsies of the patient (A) and a healthy control (B) as yellow-green fluorescence. Capillaries are indicated by arrows (bars = 5 μm).

In summary, our studies suggest that the patient carries at least one allele that results in synthesis of an LPL that is catalytically active but not adequately transported out of the adipocytes to the vascular endothelium. The low mRNA levels could be due to instability or to the presence of another mutant allele that is not transcribed into a detectable mRNA product. Studies of the LPL genome of the patient and her relatives will be required to further resolve this.

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