Missense Mutation (Gly → Glu^{188}) of Human Lipoprotein Lipase Imparting Functional Deficiency*

(Received for publication, June 29, 1989)

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Cloning and sequencing of lipoprotein lipase (LPL) cDNA prepared from the adipose tissue of a patient with classical LPL deficiency revealed a G to A transition at nucleotide 818 in all sequenced clones, leading to the substitution of glutamic acid for glycine at residue 188 of the mature protein. Hybridization of genomic DNA with allele-specific oligonucleotides confirmed that the patient was homozygous for this mutation and revealed that carrier status for this mutation among relatives of the patient was significantly associated with hypertriglyceridemia. Assay of the patient’s plasma for immunoreactive enzyme and activity demonstrated the presence of a circulating inactive enzyme protein, the concentration of which was further increased by injection of heparin. The mutant sequence was produced by oligonucleotide-directed mutagenesis, and both normal and mutant sequences were cloned into the expression vector pSVL and transfected into COS-1 cells. The normal sequence led to the in vitro expression of an enzyme that bound to heparin-Sepharose and had a specific catalytic activity similar to that of normal postheparin plasma enzyme. In contrast, the mutant enzyme expressed in vitro was catalytically inactive and displayed a lower affinity for heparin than the normal enzyme. We conclude that this single amino acid substitution leads to the in vitro expression of an inactive enzyme accounting for the manifestations of LPL deficiency noted in the patient.

Lipoprotein lipase (LPL; triacylglyceroprotein acylhydrolase, EC 3.1.1.34) is an extracellular enzyme anchored to the surface of the capillary endothelium of extrahepatic tissues by anionic interaction with heparan sulfate. The enzyme, which requires apolipoprotein C-II as a cofactor, hydrolyzes the triacylglycerols of chylomicrons and very low density lipoproteins (VLDL), thereby releasing free fatty acids for uptake into the tissues where they can either be used immediately as fuel or reesterified for storage. Thus the enzyme is essential for the distribution of energy from fatty acids among various tissues (1).

In familial LPL deficiency, a rare autosomal recessive disorder, chylomicrons accumulate in plasma as a consequence of impaired lipolysis. Subjects usually present with episodes of abdominal pain, recurrent attacks of acute pancreatitis, and eruptive skin xanthomas in early infancy or childhood; occasionally they remain asymptomatic (2, 3). Analysis of fasting plasma typically reveals type I hyperlipoproteinemia with triglyceride concentrations usually above 1500 mg/dl, normal or moderately elevated cholesterol in total plasma and in VLDL, and markedly reduced cholesterol concentrations in the low density and high density lipoprotein fractions. Absence of significant LPL activity in postheparin plasma as well as in adipose tissue establishes the diagnosis; deficiency in the cofactor apolipoprotein CII is ruled out by specific activation assays (2). The phenotype of heterozygotes for LPL deficiency remains poorly characterized (2-5).

Human LPL cDNA has been cloned and its sequence reported (6). It includes a coding region of 1425 nucleotides encoding 475 amino acids. The genomic structure of the LPL gene was elucidated while the present work was in progress (7). Although abundant in adipose, muscle, and adrenal tissues, LPL mRNA has not been detected in white blood cells (6). A recent report suggests that major genomic rearrangements of the LPL gene may account for a substantial proportion of mutations causing LPL deficiency (8).

We previously reported a patient with lipoprotein lipase deficiency and documented the occurrence of hyperlipidemia among her relatives (4). By cloning and sequencing LPL cDNA isolated from her adipose tissue, we have now determined that she is homozygous for an amino acid substitution at position 188 (Gly → Glu). Furthermore, in vitro expression of an LPL sequence including this mutation led to the production of an inactive enzyme. The identification of this point mutation has also allowed us to investigate the relationship between carrier status and hyperlipidemia in relatives of the patient.

EXPERIMENTAL PROCEDURES

Subjects—The patient, a 29-year-old female of Northern European origin, had suffered since early infancy from episodes of acute abdominal pain. Her lipid profile included marked hypertriglyceridemia (1500-2500 mg/dl), fasting chylomicronemia, and normal cholesterol concentrations. At age 19, LPL activity was nearly undetectable in her postheparin plasma and her adipose tissue, but hepatic triglyceride lipase activity and plasma cofactor activity against activator-deficient preparations were normal (4). Medical histories and blood samples were obtained from the proband and 27 of her relatives between August and September of 1988; lipoprotein analyses, apolipoprotein E phenotyping, and DNA analyses were performed on all subjects. Informed consent was obtained from all subjects and all...
... procedures were subject to prior approval by our Institutional Review Board.

Lipoprotein Analyses—Venous blood samples collected after a 12-h fast were prepared following the Lipid Research Clinics guidelines (9). Plasma lipids were characterized by a microparticle procedure described elsewhere (10).

Cholesterol in the high density lipoprotein fraction (HDLc) was measured after MgCl₂ precipitation (11). VLDL was separated from other lipoproteins by room temperature ultracentrifugation of 200 μl of plasma for 4 h at 60,000 rpm in a Beckman TL-100 tabletop centrifuge. All cholesterol and triglyceride determinations were performed with Behr reagents on an Encore II AutoAnalyzer (Baker Instruments, Allentown, PA). Apolipoprotein E isoforms were characterized by immunofocusing of VLDL (12).

Sample Handling for Lipase Analyses—Venous blood samples collected after a 12-h fast were collected in EDTA tubes before and 10 min after the intravenous injection of 60 units/kg heparin. All samples were stored at -70 °C. Before analysis, the samples (plasma or culture media) were thawed in cold water, agitated briefly to disperse the lipid particles, mixed (19:1) with 4% (w/v) sodium deoxycholate, and agitated gently on a rocking platform at room temperature for 30 min. Lipemic samples were centrifuged for 1.5 min in a microcentrifuge at 13,600 × g for 10 min, and only the infranatants were used for analysis of enzyme activity and immunoreactive mass.

Assay of Lipase Activity and Mass—Lipoprotein lipase mass was determined by a sandwich enzyme-linked immunosorbent assay using purified bovine LPL (13) as the standard. This assay recognizes immunoactive materials in a-corticosteroid binding globulin and lipoprotein lipase as well as active LPL in postheparin plasma from normal subjects (14,15). For analysis of plasma samples, the standard was prepared by adding the enzyme to LPL-free plasma which was then mixed with sodium deoxycholate as indicated above. Chromatographic fractions were analyzed using a standard diluted in 1 M NaCl, 0.1% Triton X-100, and 0.01 M sodium phosphate buffer (pH 7.5).

Affinity Chromatography on Heparin-Sepharose—Analytical hep-arin-Sepharose chromatography was performed at 0 °C using an FPLC system (Pharmacia LKB Biotechnology, Inc.) equipped with a FRAC-100 fraction collector, a HR 5/10 column, and a 10-ml Superflow for sample loading. The column (2 ml) was packed with heparin-Sepharose CL-6B, prepared as described previously (13,18), sub- merged in a cylinder of ice water, and equilibrated with 0.15 M NaCl, 0.1 M sodium phosphate buffer, and 0.01 M sodium deoxycholate buffer (pH 7.5). Samples were loaded from a loop at a flow rate of 0.2 ml/min after which the column was washed with 19.5 ml of the equilibration buffer. Elution was performed with an 8-ml linear gradient of 0.15-1 M NaCl in 0.1% (v/v) Triton X-100, 0.01 M sodium phosphate buffer (pH 7.5) at a rate of 0.65 ml/min. Finally, the column was stripped with 6 ml of 2 M NaCl in the same buffer at a rate of 0.2 ml/min. Fractions of 0.5 ml were collected throughout the procedure in tubes chilled by packing ice into the fraction collector carousel.

Isolation and Analysis of Adipose Tissue RNA—Adipose tissue (0.5 g) was obtained from the proband and a normal control individual by biopsy of the subcutaneous adipose tissue of the arm before and after an 18-h fast. Tissue was immediately placed in a cylinder of ice water, and equilibrated with 0.15 M NaCl, 0.1 M sodium phosphate buffer, and 0.01 M sodium deoxycholate buffer (pH 7.5). The tissue (200 mg) was homogenized in 1 ml of extraction buffer (50 mmol/L Tris-HCl, 300 mmol/L NaCl, 0.1% SDS, 2 mmol/L dithiothreitol, 10 mmol/L β-mercaptoethanol, and 1% sodium deoxycholate) by a Polytron (Brinkman Instruments, Madison, WI), and 200 units of mouse mammary leukemia virus reverse transcriptase (Bethesda Research Laboratories). The cDNA was recovered by ethanol precipitation after extraction with phenol/chloroform.

The oligonucleotide primers LPFL6 (5'-TATAAAGCTTCAAGGG-AAAGCTGGCCACCTG-3') and RP1607 (5'-ATCGAATTCGTTCTTTCCTGATTG-3'), synthesized on an Applied Biosystems 380A DNA synthesizer (Applied Biosystems, Foster City, CA), were used to amplify specifically a 1567-bp region (nucleotides 66-1632) of the LPL cDNA spanning its entire coding sequence. The cDNA was obtained from adipose tissue and was cloned into the polymerase chain reaction (PCR) vector (pBlueScript SK+) (Stratagene, La Jolla, CA) using PstI and EcoRI, and cloned into M13 vector. Eight independent clones were isolated, and DNA was prepared from each of them. For subcloning, DNA from each clone was further amplified (10 cycles) with primers LPFL6 and RP1PS80 (5'-TGGAGATCTTATACCATCTCTTGAGTGAAGT-3') and RP1607 to generate an 839-bp 5' cDNA fragment (nucleotides 66-904) flanked by HindIII and SalI. Mutations in the cDNA were performed by the chain termination method using an Applied Biosystems 370A DNA Sequencer as described previously (21). As a control, the cloned cDNA pLPL35 (100 pg) was amplified, cloned, and sequenced in the same manner.

Construction and Analysis of the Mutant LPL Expression Plasmid—The expression plasmid pSVL-LPL was constructed by placing the entire cDNA insert of pLPL35 downstream of the SV40 late promoter in the expression vector pSVL (Pharmacia). The 2.4-kilobase fragment was subsequently digested with HaeIII and dTTP and ligated with pSVL after digestion with HaeIII and a partial fill-in with dATP and dGTP. One clone containing the insert in the sense orientation was selected after examination of several clones by restriction enzyme digestion. The mutant expression plasmid was introduced into COS-1 cells as described previously (22). A control, the cloned cDNA pLPL35 (100 pg) was amplified, cloned, and sequenced in the same manner.

Genomic DNA Amplification and Dot-blot Hybridization—Two primers, LP790 (5'-TGGATCCGTTACACACA-3') and LP828 (5'-TGGAGATCTTCAAGGG-AAAGCTGGCCACCTG-3'), were synthesized and used to amplify, in genomic DNA of family members, a 50 bp region (nucleotides 790-845) of the LPL gene, which the proband included a G → A transition at nucleotide 818. A reaction mixture containing 1 μmol of genomic DNA was utilized to perform PCR for 25 cycles: 30 s at 94 °C, 30 s at 55 °C, and 30 s at 72 °C.

The oligonucleotide primers LP66 (5'-TATAAAGCTTCAAGGG-AAAGCTGGCCACCTG-3') and RP1607 (5'-ATCGAATTCGTTCTTTCCTGATTG-3'), synthesized on an Applied Biosystems 380A DNA synthesizer (Applied Biosystems, Foster City, CA), were used to amplify specifically a 1567-bp region (nucleotides 66-1632) of the LPL cDNA spanning its entire coding sequence. The cDNA was obtained from adipose tissue and was cloned into the polymerase chain reaction (PCR) vector (pBlueScript SK+) (Stratagene, La Jolla, CA) using PstI and EcoRI, and cloned into M13 vector. Eight independent clones were isolated, and DNA was prepared from each of them. For subcloning, DNA from each clone was further amplified (10 cycles) with primers LP66 and RP1PS80 (5'-TGGAGATCTTATACCATCTCTTGAGTGAAGT-3') and RP1607 to generate an 839-bp 5' cDNA fragment (nucleotides 66-904) flanked by HindIII and SalI. Mutations in the cDNA were performed by the chain termination method using an Applied Biosystems 370A DNA Sequencer as described previously (21). As a control, the cloned cDNA pLPL35 (100 pg) was amplified, cloned, and sequenced in the same manner.

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spotted on nylon membranes in duplicate and hybridized with $^{32}$P end-labeled oligonucleotide probes (618G, 5'-'CCAGGGGACCT-CTGGTTGA-3' or 818A, 5'-TCACCAGAGGTCCCTGG-3') corresponding, respectively, to normal or mutant cDNA. The membranes were washed at 62°C and autoradiographed as previously described (21).

RESULTS

Assay of the proband's grossly lipemic plasma for lipase activity and LPL mass was carried out after removal of the large lipid particles by centrifugation. Treatment of the plasma with deoxycholate before centrifugation prevented the simultaneous removal of adsorbed lipases, thus facilitating the demonstration of immunoreactive LPL-like material in pre- and postheparin plasma as well as hepatic triglyceride lipase in postheparin plasma (Table I). The LPL activity in the postheparin plasma of this patient was extremely low compared with the established range of 227 ± 58 nmol/min/ml (mean ± S.D.) for normal females (15), confirming our previous measurements which relied on a different assay (4). The low measured LPL activity could be due either to an analytical error or to a minor cross-reactivity of the inhibiting antibody with hepatic triglyceride lipase, resulting in a slight overestimation of LPL activity. The virtual absence of LPL activity in pre- as well as postheparin plasma, in spite of the presence of LPL-like immunoreactive material, suggested that LPL deficiency in this patient resulted from the synthesis of a dysfunctional protein.

In the absence of documented inbreeding, the proband could be either a homozygote for a single mutation or a compound heterozygote for two different mutations in the LPL gene. Southern blot analysis of the proband's genomic DNA, with pLPL35 as a probe, showed no gross alteration of LPL gene structure (data not shown). Hybridization experiments with RNA from adipose tissue indicated an apparently normal amount of full-length LPL mRNAs, 3.75 and 3.35 kilobases long (Fig. 1). Because both messages have been identified in normal subjects and the difference in size appears to result from the use of different polyadenylation signals (6), we planned to clone LPL cDNA from the proband's biopsy specimen. We amplified a 1567-bp region of LPL cDNA containing the entire coding region by means of the polymerase chain reaction (20), after synthesizing cDNA from adipose tissue total RNA (Fig. 2). Agarose gel electrophoresis of the amplified DNA showed a single DNA band of the size anticipated (data not shown).

After the amplified cDNA was cloned in M13 vector, eight independent clones were isolated and sequenced. Two additional clones amplified from pLPL35 were also sequenced as normal controls. The sequences obtained for each of the eight clones from the patient's cDNA were aligned and compared with the normal sequence. Thirteen apparent mutations were unique to individual clones; we ascribe these occasional mismatches to either sequencing artifacts or misincorporations during the amplification (24). By contrast, a single point mutation, a G → A transition at position 818 of the LPL gene, hereafter referred to as the 818A mutation, was observed in all eight clones from the patient but not in the two normal clones, in agreement with the published sequence (6). The consequence is a glycine (GGG)-to-glutamic acid (GAG) substitution at the 188th amino acid residue of the mature enzyme (Fig. 3). This result implied that either the proband was homozygous for this mutation, or she was a compound heterozygote for two distinct LPL mutant alleles and we were able to amplify and sequence mRNA species from only one of them.

Two methods were employed to examine these possibilities and to identify heterozygous carriers of the 818A mutation. As the 818A mutation abolishes a Sau96I recognition site located at position 818-822 of the normal cDNA sequence (from GGNC to AGNC), Southern blot analysis of Sau96I-digested genomic DNA was performed with an amplified 1567-bp LPL cDNA as a probe. As shown in Fig. 4, the patient and both parents presented a restriction fragment consistent with the loss of a Sau96I site. Densitometric analysis suggested that the patient was homozygous for the 818A mutation, while
Mutation in Human Lipoprotein Lipase

WILD TYPE

[Mutation sequence and amino acid comparison]

MUTANT

FIG. 3. Comparison of the nucleotide sequence of the normal to the mutant LPL cDNA surrounding position 818. Nucleotide sequences of the noncoding strands are indicated from position 805 to position 831 (right to left). Arrows point to the normal (C) and mutant (T) nucleotides. Amino acid sequence deduced from the nucleotide sequences are shown with the substitutions underlined.

her parents were heterozygous; however, the presence of another DNA fragment comigrating with the 810-bp fragment prevented a definitive inference. In a further experiment, the 818A mutation was detected directly by amplification with specific primers of a 58-bp region of genomic DNA encompassing nucleotide 818, followed by hybridization with two allele-specific oligonucleotide probes for the normal (818G) and mutant (818A) sequences. The fact that our experiments yielded the expected 58-bp fragment indicated that no intron interrupts this region of the LPL gene. Indeed, the mutation and both primer sequences lie within exon 5 of the LPL gene (7). Dot-blot hybridization of amplified genomic DNAs confirmed that the proband is homozygous for mutation 818A and that both parents are heterozygous (Fig. 5). The 818A mutation was likely to be responsible for the deficient activity of LPL in the patient because 1) she is homozygous for the 818A mutation; 2) no other mutation was detected in the entire coding region of the transcribed LPL sequence; 3) the mutation was not detected in 60 unrelated random subjects (data not shown); 4) almost no LPL enzymatic activity was detected in her adipose tissue; and 5) a significant amount of immunoreactive LPL mass was detected in postheparin plasma (Table I).

Definitive confirmation of the functional significance of the 818A mutation was established by in vitro expression of the normal and mutant LPL sequences. LPL mRNA was present at similar levels in cells transfected with vectors containing either normal or mutant LPL sequences, while such a message was undetectable after transfection with pSVL alone (Fig. 6). Cells transfected with the normal sequence (pSVL-LPL) expressed LPL activity as well as immunoreactive LPL, whereas cells transfected with the mutant sequence (pSVL-LPL818A) expressed immunoreactive material lacking significant LPL activity (Table II). COS-1 cells transfected with pSVL alone...
expressed neither activity nor immunoreactive material (data not shown). When the respective culture media were analyzed by affinity chromatography on heparin-Sepharose, the medium from cultures expressing the normal gene resulted in two peaks of immunoreactive LPL (Fig. 7A). The most retarded peak, having the highest affinity for heparin, was enzymatically active and had a specific activity (Table II) similar to that of postheparin plasma enzyme (25). The nature of the inactive material has not been investigated at this time; it could be either inactivated previously active enzyme or enzyme synthesized in an inactive form, possibly due to defective post-translational processing in this particular

FIG. 4. Hybridization of total genomic DNA after digestion with the restriction enzyme Sau961. In each lane, 5 μg of genomic DNA were electrophoresed, blotted, and hybridized with pLPL35. Lane 1, father; lane 2, patient; lane 3, mother; lanes 4 and 5, two control subjects; N, normal; M, mutant.

FIG. 5. Dot-blot hybridization analysis of the 818A mutation. The amplified genomic DNA was hybridized to two allele-specific probes for the normal and mutant sequences surrounding position 818. Representative results from the core family members of the proband are shown. Shading for symbols and identification designations are as in Fig. 8. Cloned M13 DNAs containing mutant (MU) and normal (WT) LPL sequences were amplified and hybridized as controls.

FIG. 6. Hybridization of total RNA from transfected COS-1 cells. Total RNA isolated from COS-1 cells were electrophoresed, blotted, and hybridized with pLPL25. Lane 1, cells transfected with normal LPL sequence (pSVL-LPL); lane 2, cells transfected with mutant LPL sequence (pSVL-LPL818A); lane 3, cells transfected with pSVL alone; lane 4, untransfected cells. The origins of the two major transcripts detected, of estimated sizes equal to 3900 and 3000 nucleotides, respectively, were investigated by enzymatic amplification with primers spanning either the polyadenylation signal or the VFI splice junction of pSVL after first strand cDNA synthesis of oligo(dT)-primed total RNA (data not shown). These experiments showed that a single polyadenylation signal was used and that the two species reflected the utilization of alternative acceptor sites located at nucleotides 658 and 1463 of the VFI splice junction in a manner analogous to the generation of 19 S and 16 S mRNA in SV40.

FIG. 7. Affinity chromatography on heparin-Sepharose of media from COS-1 cell cultures transfected to express (A) normal and (B) mutant (818A) LPL sequences. Media from three dishes were pooled, treated with deoxycholate, and 10 ml were loaded on the column using the 10-ml Superloop, which was cooled by submersion in ice. The column fractions were analyzed for total lipase activity and LPL concentration by immunoassay. The NaCl gradient profile was calculated by assuming an elution volume of 2.2 ml for low molecular weight buffer components. The first immunoreactive peak in panel A and the only peak in panel B had the same elution volume (36 ml) which corresponded to a concentration of 0.9 M NaCl. The second immunoreactive peak in panel A eluted at 38 ml corresponding to 1.2 M NaCl. Further experimental detail is provided under "Experimental Procedures."
TABLE II

Recovery of enzyme protein and lipase activity after affinity chromatography of COS cell media on heparin-Sepharose

| Enzyme source | Volume ml | LPL concentration ng/ml | Lipase activity mol/min/ml | Total LPL ng | Total lipase nmol/min (%)
|---------------|-----------|-------------------------|---------------------------|--------------|-------------------------|
| Starting material | | | | | |
| Normal | 10 | 83.4 | 12.6 | 834 (100) | 126 (100) |
| Mutant | 10 | 69.7 | 0.1 | 697 (100) | 1 (100) |
| Column fractions | | | | | |
| Normal | Peak I<sup>a</sup> | 2.5 | — | 744<sup>a</sup> (89) | 17 (14) |
| | Peak II<sup>b</sup> | 2.5 | — | 191<sup>b</sup> (98) | 129<sup>b</sup> (102) |
| | Total | 5 | — | 900 (115) | 146 (116) |
| Mutant | Total<sup>c</sup> | 4 | — | 462 (66) | |

<sup>a</sup>Fractions 70–74 (Fig. 7A).
<sup>b</sup>—, not calculated.
<sup>c</sup>The peak measures of total LPL protein and activity were calculated by adding up the contents of each of the contributing fractions.
<sup>d</sup>Fractions 75–79 (Fig. 7A).
<sup>e</sup>The specific enzyme activity of peak II calculates to 0.60 nmol/min/ng (600 µmol/min/mg).
<sup>f</sup>Fractions 70–77 (Fig. 7B).

DISCUSSION

The investigation of an LPL-deficient proband and her close relatives, some of whom exhibited less severe hypertriglyceridemia, has led to the identification of a missense mutation of LPL with a single amino acid substitution. This mutation, present in a double dose in the proband and resulting in the elaboration of a dysfunctional enzyme protein, was also segregating in several relatives. These findings provide new opportunities for clinical as well as biochemical investigations, whether exploring the contribution of heterozygous LPL deficiency to genetic hyperlipidemia or elucidating structure-function relationships within the LPL molecule.

Lipoprotein determinations in the patient and 27 of her relatives are reported in Fig. 8. Nine relatives of the proband presented plasma triglyceride concentrations above the 95th percentile for sex and age (26). In none of these subjects was the LDL-cholesterol concentration notably elevated. Dot-blot hybridization detected 11 heterozygous carriers of the 818A mutation (Fig. 5). The distributions of triglyceride levels were significantly different when carriers and noncarriers were compared directly by the nonparametric test of Wilcoxon using exact critical values in a two-tail test (p = 0.026). When subjects were classified as normal versus hypertriglyceridemic and Fisher's exact test was applied, ambiguity in the results reflected the arbitrariness of the classification; at a nominal level of 0.05, a 90th percentile cutoff yielded significance (p = 0.024), while a 95th percentile cutoff did not (p = 0.064).

The detection of carriers of this mutation with allele-specific oligonucleotide probes in the present pedigree, or in other pedigrees in which the same mutation may be segregating, may be a first step toward the identification of other factors, whether genetic or environmental, which lead to the
expression of hypertriglyceridemia. That as yet unidentified factors modulate the expression of the heterozygous state for LPL deficiency is supported by the observation of a normal lipid profile in some carriers, including the father of the proband (subject 3 in Fig. 8), or of hypertriglyceridemia in noncarriers (e.g. subject 13).

Lipoprotein lipase is a unique enzyme which in addition to its catalytic site (27) also has been postulated to possess domains for interfacial lipid binding (28), heparin binding (29, 30), apolipoprotein C-II binding (31), and self-association (13). The structure and precise localization of these domains have not yet been elucidated. Therefore, any naturally occurring mutation in LPL leading to a single amino acid substitution which imparts defective function may provide helpful clues for an improved understanding of structure-function relationships within this protein.

The substitution of glutamic acid for glycine at position 188, as described here, resulted in the elaboration of an apparently inactive enzyme protein which also displayed lower affinity for heparin than normal active LPL (Fig. 7). This mutation has affected the central region of homology which exhibits strong sequence conservation among lipases (32). This region harbors the domain for interfacial lipid binding and probably also the catalytic site (28, 33). Since the putative domain for heparin binding resides farther away in the carboxyl-terminal direction of the enzyme, the decreased affinity for heparin is probably due to a conformational change extending outside the region of central homology. This notion is supported by the application of the Chou-Fasman algorithm (34) which predicts that the B18A mutation should disrupt a b-turn in the secondary structure of the protein. It remains to be established whether the predicted change in conformation is present in the nascent enzyme protein or happens after the enzyme has been secreted and which other functional domains are affected. There is some evidence that the affinity of normal bovine LPL for heparin decreases when the enzyme becomes inactive (35). By analogy, the nascent mutant enzyme might be fully active and then inactivated at a much faster rate than the normal enzyme, thus leading to functional deficiency, decreased heparin binding, and circulat- ing inactive enzyme.

Acknowledgments—We thank Ray White for intellectual support and encouragement. The expert technical assistance of Bruce Dougan, Elaine Hillas, Rick Myers, David Ridinger, and Margaret Robertson, and editorial assistance by Ruth Foltz are also gratefully acknowledged.

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J. Biol. Chem. 1990, 265:5910-5916.

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