Detection and Characterization of the Heterozygote State for Lipoprotein Lipase Deficiency

Stephan P. Babirak, Per-Henrik Iverius, Wilfred Y. Fujimoto, and John D. Brunzell

Because there are no characteristic clinical or biochemical manifestations, the heterozygote state for lipoprotein lipase (LPL) deficiency has been difficult to detect. Measurements of postheparin plasma LPL activity and of LPL mass were performed in six families of probands with LPL deficiency to characterize the heterozygote state. LPL mass was measured in a sandwich enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody (5D2) that had been produced against bovine milk LPL. Thirteen obligate heterozygotes from these families had reduced LPL activity and mass below the 95th percent confidence limits of 34 normal controls, while one obligate heterozygote had LPL activity and mass between the 90th and 95th percent confidence limits. Potential heterozygotes in these families were identified as normal (n=8) or heterozygotes (n=6) by comparison to the 95th percent confidence limits of the controls. Some relatives in four of the six families exhibited mild hyperlipidemia, similar to the pattern seen in familial combined hyperlipidemia (FCHL). The hyperlipidemia segregated with the heterozygote state for LPL deficiency in these families (p<0.03). High density lipoprotein (HDL) cholesterol was significantly reduced in the heterozygotes for LPL deficiency (p<0.01). The measurement of LPL activity and mass allows identification of the heterozygote state for LPL deficiency, which is characterized by variable expressions of hyperlipidemia and reduced HDL cholesterol. These results suggest that the heterozygote state for LPL deficiency may form one subset of FCHL.


Lipoprotein lipase (LPL) deficiency is a rare clinical disorder with a frequency estimated to be less than one in a million.1,2,3 These patients have absent or very low LPL activity measurable in plasma after heparin.4 Adipose tissue LPL activity is also very low or undetectable in these patients.4,5 Classical LPL deficiency is detected in early childhood; patients show failure to thrive, colicky abdominal pain, pancreatitis, eruptive xanthomata, lactescent plasma, and other signs or symptoms of the chylomicronemia syndrome. Partial LPL deficiency syndromes have also been reported and may even be less prevalent.1,4 In patients with classical LPL deficiency, the grossly lipemic plasma results from a decreased clearance of triglyceride-rich lipoproteins.5,6 In addition, there is a marked reduction in high density lipoprotein (HDL) and low density lipoprotein (LDL), with mildly to moderately increased very low density lipoprotein (VLDL).7 These patients have normal to elevated apolipoprotein (apo) C-II levels.

Familial LPL deficiency is believed to have an autosomal recessive mode of inheritance, and consanguinity is common. The heterozygote state has been difficult to detect because postheparin enzyme activity can be low, or it may be normal.1,5,7-13 Measurement of adipose tissue LPL activity has also not been reliable for the identification of the heterozygote state.5,9 Multiple lipoprotein phenotypes have been noted in the relatives of some probands with LPL deficiency.1,7,9,10,12-16 Similar to the pattern seen in familial combined hyperlipidemia (FCHL),17 that is, hypertriglyceridemia (phenotype IV), hypercholesterolemia (phenotype IIA), or both (phenotype IIB) have been observed in successive generations. It is not known if the hyperlipidemia in the families of LPL-deficient probands is associated with the heterozygote state for LPL deficiency.5,9

The purpose of this investigation was to study LPL-deficient patients and their respective families in order to characterize the heterozygote state. When measuring LPL activity and LPL mass by enzyme-linked immunosorbent assay (ELISA), the heterozygote state for LPL deficiency in six families was characterized by reduced levels of LPL activity and mass. Relatives in four of six families with LPL-deficient patient(s) exhibited mild hyperlipidemia similar to FCHL. The hyperlipidemia segregated with the heterozygote state for LPL deficiency and was associated with reduced HDL cholesterol. Some adult heterozygotes had elevated apo B levels, which is an observation also seen in patients with FCHL. This study identifies and characterizes a genetic disorder, the heterozygote state for LPL deficiency, which may be one subset of FCHL.
Figure 1. Pedigrees from the families of eight lipoprotein lipase (LPL)-deficient patients (○). Included are the lipid phenotypes: normolipidemic (□), hypertriglyceridemic (◇), hypercholesterolemic and hypertriglyceridemic (●), and variable lipid phenotypes (◆) as reported in Table 2. Single (○) or multiple (◇) relatives were not studied. The heavy line (◆-◆) indicates consanguinity. Subject II-1 in Family 2106 had normal LPL activity and mass.

Methods

Materials
Aminopterin, hypoxanthine, thymidine 2-mercaptoethanol, caprylic acid, α-phenylenediamine dihydrochloride, 2,6,10,14-tetramethyl pentadecane (Pristane), Tween 20, heparin (porcine intestinal mucosa, 140 USP units/mg), bovine serum albumin, and horseradish peroxidase (type VI) were obtained from Sigma Chemical Company, St. Louis, Missouri. Polyethylene glycol 4000 (PEG 4000) was from E. Merck, Darmstadt, West Germany; lecithin was from Serdary Research Lab, London, Ontario; and ammonium sulfate and glycerol were from J.T. Baker Chemical Company, Phillipsburg, New Jersey. Goat anti-sera to mouse immunoglobulins and horseradish peroxidase conjugates of rabbit antibodies to goat immunoglobulins and of goat antibodies to mouse immunoglobulins were obtained from American Qualex International, Incorporated, La Mirada, California. Microtiter plates (96 wells, Nunc Immunoplate I with certificate) were purchased from Scientific Resource Associates, Bellevue, Washington. Dulbecco’s modified Eagle medium (DMEM), glutamine, and sodium pyruvate were from Gibco Laboratories, Grand Island, New York; fetal bovine serum (FBS) was from Armour Pharmaceutical Company, Kankakee, Illinois. Pooled human serum, which was heat-inactivated at 56°C for 30 minutes to remove immunoreactive LPL-like material, was obtained as described previously.20

Subject Selection
Healthy subjects (17 men, 28±4 years old, mean±SD, and 17 women, 32±9 years old) were recruited as controls. These subjects had concentrations of plasma apo B, triglyceride, and total, LDL, and HDL cholesterol between the 10th and 90th percentiles of the Lipid Research Clinic’s Prevalence Study values.21 They met the following criteria: 1) a history of good health, 2) abstinence from drugs known to affect serum lipids, 3) an age below 60 years, 4) a body weight that was stable and below 120% of ideal body weight, 5) absence of excessive regular consumption of alcohol, and 6) no history of a recent acute illness.

Eight LPL-deficient patients referred to our Lipid Clinic at the University of Washington were studied, along with 28 available family members (Figure 1, Tables 1 and 2). Consanguinity was known to be present only in Family 2106. Patients with classical LPL deficiency had: 1) fasting hypertriglyceridemia with chylomicronemia, 2) an apparent postheparin LPL activity more than three standard deviations below the mean for normal subjects, and 3) lack of secondary causes of hypertriglyceridemia. Apo C-II deficiency and a plasma inhibitor to LPL were ruled out as the cause of chylomicronemia by demonstrating the persistent lack of LPL activity after dilution of the patient’s plasma with normal plasma. In all subjects, informed consent for these studies was obtained, and procedures were approved by the Human Subjects Review Committee at the University of Washington.

Measurement of Plasma Lipoprotein Lipase Activity
All patients fasted overnight for at least 12 hours before study. An indwelling catheter was placed in an arm vein, and 10 to 20 ml of blood was collected in disodium EDTA tubes (for lipoprotein analysis) and 7 to 14 ml in lithium heparin tubes (for lipolytic activity and mass assays). A heparin bolus of 80 units/kg was injected, and 10 minutes later, 7 to 14 ml of blood was collected in lithium heparin tubes for the determination of LPL activity and mass. Samples were cooled on ice, and the plasma was separated after centrifugation (3000 g, 10 minutes at 4°C). The plasma samples were then stored at −70°C before assay.

Total postheparin plasma lipolytic activity was measured as previously reported.20 Aliquots of the enzyme
source were incubated with the substrate, tri-1-14C-oleate, for 60 minutes at 37°C, and the liberated free fatty acids were extracted and counted. Activity is expressed as nanomoles of fatty acids released per minute per millilitre of plasma. To determine hepatic triglyceride lipase (HTGL) and LPL activity, LPL was selectively blocked by the monoclonal antibody, 5D2, obtained as described below. This monoclonal antibody binds LPL and inhibits human, bovine, and rabbit LPL activity. The 5D2 monoclonal antibody completely blocked activity of human adipose tissue LPL. LPL activity was calculated as the activity inhibited by this antibody when added to plasma. On each assay occasion, a bovine milk lipase standard and a human this antibody when added to plasma. On each assay occasion, a bovine milk lipase standard and a human

**Preparation of the 5D2 Antibody**

Bovine milk LPL was purified and yielded one band on sodium dodecyl sulfate polyacrylamide electrophoresis.22 Purified LPL (250 μg/ml) was emulsified with an equal volume of Freund's complete adjuvant, and aliquots (25 μg of LPL) were administered subcutaneously to specific pathogen-free female Balb/CJ mice (Jackson Laboratories, Bar Harbor, ME), with intraperitoneal booster doses at 5 and 8 weeks plus intravenously (50 μg In 0.1 ml of sterile saline) at 11 to 15 weeks. Four days after the last antigen injection, splenocytes were harvested and were fused with an equal number (5 x 10^5) of P3X63 Ag 8.653 mouse plasmacytoma cells (American Type Culture Collection, Rockville, MD) by using PEG 4000. The fused cells were maintained on feeder macrophages obtained by peritoneal lavage of mice in selection medium (DMEM with 2 mM glutamine, 1 mM sodium pyruvate, 50 μM 2-mercaptoethanol, and 10% FBS) containing hypoxanthine, aminopterin, and thymidine. The clones that produced antibodies reacting with LPL were identified by screening the culture media with an ELISA.24 Briefly, bovine LPL (1 μg/ml) in phosphate-buffered saline (PBS) was adsorbed at 37°C for 1 hour on microtiter plates (100 μl/well). The wells were rinsed three times with PBST [PBS containing 15% glycerol (vol/vol), 1% Tween 20 (vol/ vol), and 1 mg/ml sodium heparin], then incubated with tissue culture media (3- to 100-fold dilution) in PBST with horseradish peroxidase-labeled goat antimouse IgG (2000-fold dilution). Peroxidase activity was assayed as described by Engvall.25 One antibody (5D2) identified as the most potent inhibitor of LPL activity was grown in ascites tumors from mice primed with Pristane. The 5D2 antibody was then purified from ascites fluid by sequential caprylic acid and ammonium sulfate precipitation with 5.7 to 13.8 mg of IgG recovered per millilitre of ascites fluid. An antibody dose–response inhibition curve for LPL by the 5D2 antibody demonstrated that the 5D2 antibody completely inhibited catalytic activity at 10^-5 dilution of ascites fluid. Despite the homology between LPL and hepatic lipase, the 5D2 antibody was specific for LPL and did not inhibit a partially purified hepatic lipase preparation obtained after heparin agarose chromatography. The immunoglobulin subclass of 5D2 was identified as IgG by EUSA with subclass-specific goat antimouse immunoglobulin sera and horseradish peroxidase-labeled rabbit anti-goat IgG antibodies.

**Enzyme-linked Immunoassay for Lipoprotein Lipase Mass**

The 5D2 antibody (0.4 μg/ml in 200 μl of PBS) was incubated in microtiter wells at 37°C for 4 hours. Plates were then rinsed three times with PBST and stored at 4°C for up to 1 week. Purified bovine LPL standards were stored at -70°C and were mixed with pooled human plasma at 4°C before assay. Standards (0 to 100 ng) were run in quadruplicate, were incubated in microtiter wells (200 μl/well pretreated with antibody) at 4°C for 20 hours, and then were rinsed four times with PBST at room temperature. 5D2 antibody was conjugated to horseradish peroxidase with 20% Tween 20 (vol/vol) in PBS, and was added (200 μl/well) to the plates and then incubated at room temperature for 3 hours. The

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**Table 1. Clinical Data for Lipoprotein Lipase-Deficient Patients**

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<tr>
<th>Family</th>
<th>Pedigree</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>TG (mg/dl)</th>
<th>TC (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
<th>Apo B (mg/dl)</th>
<th>LPL Act (nmol/ min/ml)</th>
<th>LPL Mass (ng/ml)</th>
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<td>13</td>
<td>303</td>
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<td>16</td>
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*Controls*  

Controls*  

**Total**  

**Pedigree**  

2105 II-2 21 F 1995 361 24 14 323 98 0 35  

2106 II-2 40 M 2040 287 19 12 256 98 0 38  

2107 III-1 13 F 3690 337 - - - 74 5 59  

2107 III-2 7 F 2400 222 - - - 50 3 28  

2108 III-1 <1 F >1575 474 - - - 4 5 50  

2109 II-1 5 F 2780 258 - - - 18 2  

2110 III-2 1 M 776 185 9 913 94 24 103  

2110 III-3 7 M 3408 348 32 13 303 93 16 99  

220±60 212±64  

212±60 179±50  

220±59 196±59  

**TG = total triglyceride, TC = total cholesterol, LDL-C = low density lipoprotein cholesterol, HDL-C = high density lipoprotein cholesterol, VLDL-C = very low density lipoprotein cholesterol, Apo B = apolipoprotein B, LPL Act = lipoprotein lipase activity, LPL Mass = lipoprotein lipase mass.**
Table 2. Clinical Data of Relatives

<table>
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<th>Family</th>
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<th>Age (yrs)</th>
<th>Sex</th>
<th>TG* (mg/dl)</th>
<th>TC* (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>Apo B (mg/dl)</th>
<th>Phenotype</th>
<th>Act (nmol/min/ml)</th>
<th>Mass (ng/ml)</th>
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<td></td>
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<td></td>
</tr>
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<td>96</td>
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<td>101</td>
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</table>

Potential heterozygotes

| Normal LPL | | | | | | | | | | |
| 2105 | II-1 | 22 | M | 46 | 173 | 108 | 55 | 89 | NL | 270 | 202 |
| 2105 | II-3 | 7  | F | 33 | 188 | 109 | 77 | 88 | NL | 244 | 182 |
| 2106 | III-4 | 11 | M | 43 | 158 | 87  | 62 | 76 | NL | 159 | 126 |
| 2107 | I-1 | 70 | F | 133 | 241 | -   | -  | -  | NL | 234 | 281 |
| 2107 | III-3 | 5  | F | 68 | 141 | -   | -  | -  | NL | 213 | 169 |

| Detected heterozygotes | | | | | | | | | | |
| 2106 | II-3 | 40 | M | 118 | 181 | 124 | 33 | 130 | NL,IV | 72 | 62 |
| 2106 | II-4 | 30 | F | 84 | 164 | 105 | 42 | 88 | NL | 123 | 193 |
| 2106 | III-3 | 12 | F | 90 | 151 | 81  | 52 | 61 | NL | 46 | 39 |
| 2107 | II-1 | 29 | M | 242 | 231 | -   | -  | 135 | NL-II-B | 122 | 186 |

| Controls | | | | | | | | | | |
| F | | | | | | | | | | |
| M | | | | | | | | | | |

Totals

| 220±59 | 196±59 |

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plates were then rinsed five times with PBST, and peroxidase activity was measured by absorbance at 492 nm. Standards of bovine or human LPL were linear to 100 ng/ml with a correlation coefficient typically greater than 0.97. A close correspondence was found between regression lines of bovine and human LPL, indicating that the ELISA detects enzyme from both sources with similar sensitivity. Heat inactivation of human enzyme demonstrated that enzyme mass was lost in parallel with enzyme activity.

LPL mass in patient samples was determined by using the ELISA described above and was calculated from the standard curve by using purified bovine LPL. LPL mass was measured in preheparin and postheparin plasma. The incremental LPL mass released into the plasma after heparin administration was calculated by subtracting preheparin from postheparin plasma mass. Samples were measured in octuplicate, and the interassay variation was 7.8% with an intra-assay variation of 2.8%. A human postheparin plasma lipase standard was included to correct for interassay variation.

Other Procedures

Total cholesterol, LDL cholesterol, HDL cholesterol, VLDL cholesterol, and total triglyceride were measured according to modified procedures of the Lipid Research Clinic.\(^\text{27}\) Apo B was measured by radioimmunoassay.\(^\text{28}\) Hyperlipidemia was diagnosed if a subject's age-adjusted plasma level was above the 95th percentile for total cholesterol (type IIa) or total triglyceride (type IV), or if both total cholesterol and total triglyceride were above the 90th percentile (type IIb). The control population for lipids was defined as the Lipid Research Clinic control population matched for age and sex.\(^\text{21}\) The control population for apo B was defined from a Seattle control population.\(^\text{29}\)

Statistical Methods

Data were analyzed by: 1) unpaired Student's t test, 2) regression analysis, 3) Fisher's exact test, and 4) \(\chi^2\) analysis. The results are expressed as means ± SD. The ellipse that describes the 95th percentile confidence limits of activity and mass for the controls was calculated from the mean and variance of the enzyme activity and the enzyme mass plus the covariance between them as described by Zelen and Severo.\(^\text{30}\)

Results

Lipoprotein Lipase Activity and Mass in Postheparin Plasma from Control Subjects

LPL activity was similar in the 17 normolipidemic men (212±60 nmol/min/ml) and 17 women (227±58) and the values were, therefore, combined (220±59). Similarly, values for LPL mass in the men (179±50 ng/ml) and women (212±64) were combined (196±59). The postheparin plasma activity correlated with the incremental enzyme mass in these 34 controls (Figure 2) \((r=0.83, p<0.001)\), indicating that the specific activity of the postheparin plasma enzyme of these normolipidemic subjects was fairly uniform \((1.15±0.20 \text{ nmol/min/ng, coefficient of variation}=17\%)\). This high correlation between activity and mass, which appears in postheparin plasma, adds strength to the concept that the ELISA measures releasable enzyme mass, since enzyme activity is determined by independent means.

Detection of Heterozygote State

Most LPL-deficient patients have absent or low LPL activity and incremental LPL mass (Figure 3). However, some patients had low activity and detectable enzyme mass, a finding that is consistent with a defective enzyme protein. Since LPL deficiency is an autosomal recessive disorder, obligate heterozygotes are defined as the parents or the offspring of an LPL-deficient patient. The obligate heterozygotes from six families had reduced LPL and fell below the 95th percentile confidence limits for LPL activity and mass compared to the controls, except for one
obligate heterozygote (Family 2105:1-2), whose LPL fell between the 90th and 95th percent confidence limits (Figure 4). When comparing only LPL activity, there was some overlap between the heterozygotes and normal controls, which may explain why previous attempts at detection of the heterozygote state were unsuccessful. However, by using both LPL activity and mass, the heterozygotes appeared to have abnormal LPL because they fell outside these 95% confidence limits.

From these six families, 14 other family members who were potential heterozygotes (i.e., they had a 50% chance of inheriting one abnormal gene for LPL deficiency) were sampled. With the combined measurement of LPL activity and LPL mass, eight of the 14 potential heterozygotes had normal LPL, while six were classified as heterozygotes because they had reduced LPL and fell below the 95th percent confidence limits of normal (Figure 5). In Family 2110, one would expect that a paternal grandparent of the proband should be a heterozygote. Since both grandparents have normal LPL, either we are unable to detect the heterozygote, or the first affected individual (II-1) had abnormal LPL due to a new mutation in this family.

**Characterization of the Heterozygote State**

Plasma lipids were analyzed in the relatives from six families (Figure 1 and Table 2). In four of the six families, mild hyperlipidemia was present in ten of the relatives of LPL-deficient patients. An example of this mild hyperlipidemia is depicted in Family 2106. The LPL-deficient patient (II-2) has a son (III-2), a brother (II-3), and a father (I-1) with variable hyperlipidemia, as well as a son (III-1) with elevated serum cholesterol and triglyceride. Three of six affected first-degree relatives were normolipidemic on one occasion and hypertriglyceridemic (I-1, II-3) or hypertriglyceridemic and hypercholesterolemic (III-2) on another occasion. Since the proband's parents were cousins, the mother and father most likely contributed the same mutant gene; therefore, different gene defects cannot account for this variability. When measured, the LDL cholesterol was above the 90th percentile in the hypercholesterolemic heterozygotes (n=3), indicating that the increase in total cholesterol could be accounted for in the LDL fraction. The hyperlipidemia segregates with the heterozygote state in these families. Ten of 20 heterozygotes were hyperlipidemic, while all relatives with normal LPL (n=8) were normolipidemic (p<0.03).

LPL-deficient probands have markedly reduced HDL cholesterol (Table 1). The heterozygotes for LPL deficiency were also characterized in this study by reduced HDL cholesterol (Table 2). In 10 of 17 heterozygotes, the HDL cholesterol was below the 25th percentile, while only three of 17 heterozygotes were above the 50th percentile. Quartile x² analysis showed that HDL cholesterol was reduced in the LPL-deficient heterozygotes compared to the Lipid Research Clinic's control population (p<0.01). Plasma apo B levels were above the 90th percentile in six of the 16 adult heterozygotes, while one of the relatives with normal LPL had an apo B above the 90th percentile.

**Discussion**

Since LPL deficiency is a rare clinical disorder having a frequency approximately equal to the homozygote state of familial hypercholesterolemia (1 in 1 million),⁹ the heterozygote state of each disorder may also have similar frequencies (approximately 1 in 500). It has been difficult to characterize the heterozygote state for LPL deficiency since both the nonspecific measurement of postheparin lipolytic activity and the specific measurement of LPL activity have been unreliable in identifying heterozygotes.⁷–¹² It has been reported⁶ that adipose tissue LPL activity from subcutaneous fat biopsies is depressed to about 50% of normal in the parents and healthy sibs of one LPL-deficient subject in a small nuclear family. However, measurement of adipose tissue LPL activity could not identify heterozygotes in another family with an extended pedigree.⁹ These observations would appear to contradict the expectation that enzyme levels should be reduced to about 50% of normal in heterozygotes for an autosomal recessive disorder such as LPL deficiency. However, when both LPL activity and mass are used to compare obligate heterozygotes for LPL deficiency to normal controls, it becomes apparent that the heterozygotes have reduced LPL and fall below the 95th percent confidence limits of the control subjects. Using this technique, we can now identify heterozygote individuals in families of patients with LPL deficiency. In
addition, this technique has allowed us to initially characterize the heterozygote state.

Although hyperlipidemia is not observed in all families of LPL-deficient probands,\textsuperscript{7,8,10,12} this study demonstrates that the mild variable hyperlipidemia observed in some families of LPL-deficient probands segregates with the heterozygote state for LPL deficiency. Not all of the heterozygotes had hyperlipidemia, and the heterozygotes who had their lipids measured on more than one occasion demonstrated that their lipid profile could vary from one abnormal phenotype to another or could be normal. This pattern of hyperlipidemia seen in successive generations, which varies within and between individuals, has been reported by several investigators\textsuperscript{9,10,12,18} in other families with an LPL-deficient proband and is similar to that pattern seen in the families with FCHL. Some adult heterozygotes for LPL deficiency had elevated apo B levels, which also makes these heterozygotes appear similar to patients with FCHL. Wilson et al.\textsuperscript{9} proposed a second genetic defect to explain the hyperlipidemia in the family of one LPL-deficient patient; however, LPL-deficient heterozygotes could not be reliably identified in that study. The present study suggests that the lipoprotein abnormalities and the heterozygote state for LPL deficiency are due to a common gene defect in these families with an LPL-deficient proband.

One interesting feature observed in this study is the expression of hyperlipidemia in some children who are heterozygotes for LPL deficiency. Other studies have also documented hyperlipidemia in children from families with an LPL-deficient proband.\textsuperscript{7,8,12,13,14,18} Sniderman et al.\textsuperscript{31} reported that some children from families with FCHL had hyperlipidemia, elevated levels of apo B, or both, while Goldstein et al.\textsuperscript{32} suggested that hyperlipidemia in FCHL is not usually manifest until adulthood. This difference in the age of onset of hyperlipidemia in various populations with FCHL is compatible with the heterogeneity of this disorder. The families with FCHL in whom the children are affected may be a subset of those who are heterozygotes for LPL deficiency.

Hypertriglyceridemia in those heterozygous for LPL deficiency can be explained on the basis of a defective clearance of serum triglycerides, which is probably secondary to abnormal or reduced LPL, as noted by Hartan et al.\textsuperscript{5} It has been reported that a decrease in LPL is associated with a decrease in VLDL fractional catabolism,\textsuperscript{33} which would explain the hypertriglyceridemia we saw. The decrease in HDL cholesterol would be explained by the reduced hydrolysis of VLDL triglyceride, resulting in a decreased contribution of VLDL surface components to HDL.\textsuperscript{33} The combined presence of hypertriglyceridemia and hypercholesterolemia, which has also been reported in heterozygotes for LPL deficiency, could be related to a defective clearance of serum triglyceride leading to an increase in precursor lipoproteins for LDL synthesis.

The occurrence of hypercholesterolemia due to elevated LDL levels alone is more difficult to explain but has been a consistent observation in these families. There are several possible mechanisms to account for this observation. LPL appears to facilitate the uptake of free cholesterol during chylomicron hydrolysis in perfused mammmary gland and adipose tissue.\textsuperscript{34,35} The transfer of cholesteryl ester from plasma lipoproteins to cells also appears more active in those cells that produce LPL.\textsuperscript{36-40} In perfused rat heart, cultured rat heart cells, and cultured preadipocytes, chylomicron cholesteryl ester uptake required LPL and was markedly reduced after LPL was displaced from the capillary endothelium or the cell surface by heparin. This uptake of cholesteryl ester was distinct from the LDL receptor pathway and did not require that triglyceride hydrolysis be functional. Several additional studies suggest that the increased transmembrane transport of cholesteryl ester in cells with LPL is somehow catalyzed by the enzyme, possibly by providing an extensive surface area for the removal and metabolism of phospholipid and cholesteryl ester.\textsuperscript{40}

The relationship of FCHL to the heterozygote state for LPL deficiency remains to be elucidated. Previous studies have demonstrated that LPL activity appears to be normal in patients with FCHL.\textsuperscript{41} Goldberg et al.\textsuperscript{42} studied five patients with FCHL who had normal LPL activity in adipose tissue during both the fasting and the postprandial state. Beil et al.\textsuperscript{43} studied 11 patients with FCHL who demonstrated normal postheparin LPL activity as a group, but three of these patients had reduced postheparin LPL activity as compared to normal controls. A recent study\textsuperscript{44} of six kindreds with FCHL demonstrated that LPL activity was not different between normotriglyceridemic and hypertriglyceridemic relatives, as well as normal controls. The proportion of patients with FCHL who are actually heterozygous for LPL deficiency remains to be determined. Previous studies have not distinguished between patients with FCHL who are and those who are not heterozygotes for LPL deficiency for several reasons: 1) the heterozygote state cannot be identified clinically, 2) biochemical markers for the heterozygote state were previously unreliable, and 3) FCHL is a relatively common disorder (1% to 2% of the population), while the heterozygote state is much less common (1:500); thus, any investigation has a selection bias against the heterozygote state. Now that there is a method for the detection of heterozygotes for LPL deficiency, it may be possible to identify what proportion of FCHL patients actually are heterozygotes for LPL deficiency.

In summary, a method that can reliably detect the heterozygote state for LPL deficiency is described. The heterozygotes had reduced LPL activity and mass, less than the 95th percent confidence limits of normal controls. Some of the heterozygotes had a mild variable hyperlipidemia, reduced HDL cholesterol, and may or may not have had elevated apo B levels, which make these patients clinically similar or indistinguishable from patients with FCHL. This study identified one genetic disorder, the heterozygote state for LPL deficiency, as a subset of FCHL. The prevalence of heterozygotes for LPL deficiency among patients with FCHL remains to be determined.

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References


ester by cultured cells. Biochim Biophys Acta 1984; 795:47–59


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S P Babirak, P H Iverius, W Y Fujimoto and J D Brunzell

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