Hemolysis in Primary Lipoprotein Lipase Deficiency

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A slight to moderate hemolysis is often present in plasma from patients with primary lipoprotein lipase (LPL) deficiency. To determine the nature of this hemolysis, we measured erythrocyte hypo-osmotic fragility, plasma free hemoglobin, and phospholipid composition in 26 patients with primary LPL deficiency and 21 unrelated controls. In some patients, these investigations were completed by erythrocyte cytoskeletal protein determinations and abdominal echography. Osmotic fragility was similar between control subjects and patients. However, there was a significantly increased concentration of plasma free hemoglobin in primary LPL deficiency (0.282 ± 0.331 *v* 0.048 ± 0.038 g/L in controls, $P < .005$). In LPL-deficient patients, an increase of plasma lysophosphatidylcholine concentration (12.6% ± 5.8% *v* 6.4% ± 1.9% in controls, $P < .0001$) was also found. The protein composition of the erythrocyte membrane skeleton was abnormal in some LPL-deficient patients and splenomegaly was present in 12, but these abnormalities did not correlate with plasma free hemoglobin levels. Bilirubin and haptoglobin levels were also within physiologic ranges in these patients, suggesting that the observed hemolysis did not result from hypersplenism. It appears likely that the accumulation of lysophosphatidylcholine was due to an impairment in the reverse metabolic pathway converting lysophosphatidylcholine back to phosphatidylcholine. Collectively, these data, along with a positive correlation between plasma free hemoglobin and lysophosphatidylcholine levels ($r = .58$, $P = .0001$), suggest that the hemolysis observed in primary LPL deficiency is mediated to some extent by the abnormally elevated concentration of lysophosphatidylcholine.

PRIMARY PLASMA lipoprotein lipase (LPL) deficiency is an autosomal recessive disorder characterized by the absence of LPL activity in postheparin plasma and severe chylomicronemia. The incidence of primary LPL deficiency is one case per million. However, in the province of Quebéc, the incidence reaches 200 cases per million in some areas.[1] Most LPL-deficient patients from western Quebéc were found to have a missense mutation at residue 188 of the LPL gene,[2] whereas most LPL-deficient patients from the northeastern part of the province were found to have a missense mutation at residue 207 of the LPL gene.[3] In fasting homozygotes, these mutations resulted in marked hypertriglyceridemia due to defective catabolism of plasma chylomicrons. In 56 French-Canadian patients suffering from primary LPL deficiency, fasting triglyceride and cholesterol levels have been found to be greater than 27 and 7 mmol/L, respectively. The bulk of these lipids is contained within the chylomicrons, whereas low-density lipoprotein and high-density lipoprotein particles are greatly reduced. Clinical manifestations associated with this form of primary chylomicronemia include abdominal pain with or without pancreatitis, hepatomegaly, splenomegaly, eruptive xanthomas, and lipemia retinalis.[1]

Frohlich and Godin[4] reported decreased membrane cholesterol concentration, modification of phospholipid distribution, and increased osmotic fragility in erythrocytes from one patient with primary LPL deficiency. A number of inherited metabolic diseases in which plasma lipid alterations affect erythrocyte lipid composition have been reported. For instance, in abetalipoproteinemia and hypobetalipoproteinemia, increased erythrocyte membrane cholesterol concentration and modifications in the distribution of major phospholipids of the cell membrane result in acanthocytosis.[5,6] In analphalipoproteinemia such as that found in Tangier's disease, decreased membrane cholesterol concentration and variations in phospholipid distribution cause stomatocytosis, anisocytosis, and increased erythrocyte osmotic fragility.[4,7] Patients with lecithin:cholesterol acyltransferase (LCAT) deficiency show high plasma concentrations of unesterified cholesterol and phosphatidylcholine, increased membrane cholesterol, modifications in phospholipid profiles, and formation of target cells that display decreased osmotic fragility.[8,9] Alterations of the erythrocyte cytoskeleton have also been shown to affect erythrocyte morphology and function.[10]

In a previous study of 16 French-Canadian patients with primary LPL deficiency, we observed a number of alterations in plasma and erythrocyte membrane lipids.[11] Three of them were of particular interest: (1) modifications in the plasma phospholipid distribution, with a significant increase (twofold) in lysophosphatidylcholine concentration; (2) modifications in the phospholipid profile of the erythrocyte membrane; and (3) a decrease in the concentration of erythrocyte membrane cholesterol. The presence in plasma of the cytolytic lysophosphatidylcholine,[11] the lipid modifications of the erythrocyte membrane,[4,11] and the presence of splenomegaly in 40% of the adult patients[1] have led us to investigate the hemoglobinemia, erythrocyte cytoskeletal proteins, and osmotic fragility of 26 patients with familial LPL deficiency. These investigations have demonstrated an elevated plasma free hemoglobin concentration without clinical signs of intravascular or extravascular hemolysis.

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Furthermore, erythrocytes from LPL-deficient patients did not exhibit any increased susceptibility to in vitro hypo-osmotic lysis.

SUBJECTS AND METHODS

Twenty-six patients (14 men and 12 women) with clinically documented primary LPL deficiency were compared with 21 unrelated controls (18 men and 3 women) who showed no signs of LPL activity impairment. Of the 21 control subjects, two presented with endogenous hypertriglyceridemia without chylomicronemia (total plasma triglyceride levels, 3.53 and 6.14 mmol/L). The 26 LPL-deficient patients are part of a previously described cohort of French-Canadians suffering from complete LPL deficiency.[1] Plasma postheparin LPL and hepatic lipase activities were determined in the LPL-deficient group[12] and compared with activities in a second group of 26 normolipidemic volunteers (11 women and 15 men). To confirm the primary LPL deficiency diagnosis, DNA analyses were performed and known mutations of the LPL gene were identified. Two patients were homozygous for mutation 188,[2] 18 were homozygous for mutation 207,[3] and five were found to be compound heterozygotes (188/207). Another patient without plasma LPL activity was characterized by a missense mutation at residue 250 on one allele and a yet-undetermined mutation on the other allele.[13] Eighteen of 21 control subjects were investigated and found to be normal regarding known LPL mutations.

All our primary LPL-deficient patients were free-living individuals who were invited to follow an appropriate diet for their condition, which included a medium-chain triglyceride supplement (MCT oil; Mead Johnson, Ottawa, Canada). Echographic determination of spleen volume was performed during routine clinical examination of LPL-deficient patients.

Plasma Lipid Analysis

After a 12-hour fast, blood samples were collected by venipuncture into tubes containing EDTA. Fresh blood was centrifuged at 1,400 × *g* and 4°C for 15 minutes. Plasma was separated from erythrocytes, and the buffy coat was removed. Plasma cholesterol, free cholesterol, triglyceride, and phospholipid levels were determined enzymatically using an automated RA-1000 analyzer from Technicon Instruments (Tarrytown, NY).[11]

Plasma lipoprotein fractions were prepared by the combined use of ultracentrifugation and heparin-manganese precipitation.[14] In LPL-deficient patients, chylomicron-free plasma was prepared by ultracentrifugation of total plasma at 80,000 × *g* for 30 minutes using a swinging-bucket rotor (SW-40, Beckman Instruments, Palo Alto, CA). Chylomicrons were then stored at −20°C for subsequent phospholipid analysis.

Characterization of Plasma Phospholipids

Plasma and chylomicron phospholipids were extracted by partition in chloroform/methanol. Lipid extracts obtained were fractionated by one-dimensional thin-layer chromatography on precoated 0.25-mm layers of silica gel H plates (Analtech, Newark, NJ). Chromatographic plates were heat-activated for 1 hour at 120°C before use. Lipid extracts were quantitatively transferred, spotted onto thin-layer chromatography plates, and chromatographed in chloroform/methanol/acetic acid/water (100:45:20:7). This allowed complete fractionation of major phospholipids (sphingomyelin, phosphatidylcholine, phosphatidylinositol, phosphatidylserine, and phosphatidylethanolamine), as well as lysophosphatidylcholine. Phospholipid bands were visualized on chromatograms by brief exposure to iodine vapor and then collected by scraping. Quantitative determinations of lipid phosphorus were performed as previously described.[11] Since more than 97% of the phosphorus was located in previously mentioned bands, no further resolution of minor phospholipid fractions (cardiolipin, phosphatidylglycerol, and phosphatidic acid) was undertaken.

Erythrocyte Protein Analysis

Erythrocyte ghosts were prepared as described by Hanahan and Ekholm.[15] Briefly, erythrocytes were suspended in a 0.9% NaCl solution to obtain a final hematocrit of 50%. They were then washed in a chilled solution of 0.011 mol/L Tris buffer (pH 7.6) and centrifuged at 20,000 × *g* for 40 minutes. Four washes of the erythrocytes in the Tris buffer were necessary to completely release hemoglobin. Before storage at −80°C, the protein concentration of resulting ghost membranes was measured using bicinchoninic acid.[16]

Erythrocyte ghost membranes were thawed at 4°C, mixed with an equal volume of twice-concentrated sample buffer (10 mmol/L Tris, pH 8.0, 1.0 mmol/L EDTA, 3.3% sodium dodecyl sulfate, 10% glycerol, and 40 mmol/L dithiothreitol), and heated at 90°C for 2 minutes. The acrylamide to bisacrylamide ratio in the stock solution was 39.2:0.8 (wt/wt). Protein samples from patients and control subjects were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis according to the technique reported by Laemmli[17] using a 7% to 15% linear gradient in the separating gel to increase the degree of resolution of proteins of interest in this study. Electrophoresis was performed at 150V for 60 minutes in a Miniprotean II electrophoresis system (BioRad, Mississauga, Canada). Gels were stained with Coomassie Blue R-250. Purified human spectrin and specific muscle proteins of known molecular weight were used as molecular weight standards.

Plasma Free Hemoglobin Measurements

Plasma free hemoglobin level was measured using orthotolidin.[18] Hemoglobin standards were obtained from Fisher Scientific (Québec City, Canada). In LPL-deficient patients, plasma samples were centrifuged at 80,000 × *g* before analysis to remove chylomicrons that could interfere with the spectrophotometric assays.

To examine the hemolytic susceptibility of erythrocytes exposed to chylomicronemic plasma, erythrocytes collected by venipuncture into EDTA-containing tubes from normolipidemic subjects were incubated with plasma obtained from LPL-deficient patients. Fresh blood samples were obtained as previously described from six LPL-deficient patients and 12 control subjects. Control erythrocytes were isolated from whole blood after a 10-minute centrifugation (1,400 × *g*) at 4°C and immediately incubated with normal or chylomicronemic plasma for a 30-minute period at room temperature under gentle agitation. Blood samples were then recentrifuged for 10 minutes at 1,400 × *g* and 4°C to determine plasma free hemoglobin concentration. A similar experiment was also conducted using chylomicron-free plasma from three LPL-deficient patients and erythrocytes from 10 control subjects to ascertain the hemolytic potential of chylomicron-free plasma.

Osmotic Fragility Measurements

Fasting blood samples were collected by venipuncture into heparinized tubes. A 30-μL aliquot of whole blood was added to 3.0 mL of a phosphate-buffered NaCl solution. Blood samples of each patient and control subject were submitted to different osmotic forces ranging from 0 to 400 mOsm. All samples were gently mixed and left standing for 30 minutes and then centrifuged at 1,000 × *g* for 5 minutes. In LPL-deficient patients, 1.5 mL supernatant was removed and submitted to another centrifugation for 30 minutes at 15,000 × *g* to remove chylomicrons. Absorption of the chylomicron-free supernatant was measured at 545 nm.[18] The

percentage of hemolysis was calculated relative to the 100% value obtained in distilled water. Osmotic fragility curve equations were obtained by fitting the data to a logistic regression model. The point of 50% hemolysis and the percentage of hemolysis at 300 mOsm were calculated from the regression curves.

Statistical Analyses

Regression curves describing osmotic fragility were obtained using the nonlinear regression option of the STATGRAPHICS statistical software.[19] All other statistical analyses were performed on the SAS statistical system (Cary, NC). An ANOVA was used to evaluate differences between groups. Results were considered statistically significant at $P \leq .05$.

RESULTS

Plasma Lipid Analyses

LPL-deficient patients were hyperlipidemic, as shown by their elevated concentrations of plasma triglycerides and phospholipids (Table 1). They also had elevated concentrations of plasma free cholesterol. Lipoprotein fractionation showed that most of the cholesterol and triglycerides in LPL-deficient patients were located in the chylomicron fraction. Plasma postheparin LPL activity was not detectable in LPL-deficient patients, whereas hepatic lipase activity was found to be similar to that of a group of normal volunteers (22 ± 13 nmol/mL/min in LPL-deficient women *v* 23 ± 10 in volunteer women and 42 ± 27 in LPL-deficient men *v* 51 ± 18 in volunteer men).

Chylomicron-free plasma of LPL-deficient patients showed significant differences in phospholipid distribution as compared with controls (Table 2). Although phosphatidylcholine, the major plasma phospholipid, and phosphatidylethanolamine were of comparable levels in both groups, sphingomyelin was significantly decreased and lysophosphatidylcholine, phosphatidylinositol, and phosphatidylserine were significantly increased in LPL-deficient patients. Chylomicron phospholipid distribution is listed in Table 2. The highest lysophosphatidylcholine concentrations were found in chylomicron-free plasma.

Table 1. Plasma Lipid Profiles (mmol/L) in Primary LPL Deficiency

	Control	LPL-Deficient	*P*
Age (yr)	28 ± 8 (21)	29 ± 14 (26)	NS
Plasma			
Total cholesterol	4.79 ± 0.91 (21)	7.05 ± 5.63 (26)	NS
Free cholesterol	1.23 ± 0.25 (14)	2.68 ± 1.85 (16)	.0074
Triglycerides	1.25 ± 1.31 (21)	24.77 ± 16.79 (26)	.0001
Phospholipids	2.36 ± 0.28 (16)	3.86 ± 2.76 (23)	.0371
Chylomicrons			
Total cholesterol	—	5.00 ± 3.94 (19)	—
Triglycerides	—	20.83 ± 12.15 (19)	—
VLDL			
Total cholesterol	0.56 ± 0.78 (21)	1.42 ± 2.72 (20)	NS
Triglycerides	0.80 ± 0.77 (21)	3.77 ± 6.71 (20)	NS
LDL			
Total cholesterol	3.24 ± 0.69 (21)	0.56 ± 0.19 (20)	.0001
Triglycerides	0.13 ± 0.15 (21)	0.37 ± 0.24 (20)	.0003
HDL			
Total cholesterol	1.12 ± 0.23 (21)	0.34 ± 0.07 (20)	.0001
Triglycerides	0.24 ± 0.05 (21)	0.34 ± 0.09 (20)	.0001

NOTE. Values are the mean ± SD. NS, $P > .05$. Number of patients is given in parentheses for each measurement.

Abbreviations: VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Erythrocyte Protein Analyses

To minimize proteolytic activity, all samples were used immediately after thawing. Membrane proteins studied in three of eight patients (randomly chosen from 26 used for this study) showed comparable electrophoretic patterns (Fig 1B). However, for the remaining five patients (Fig 1A), considerable reductions in some protein fractions were obtained following electrophoretic analysis. The protein doublet 2.2a and 2.3a was considerably reduced in samples from four patients and completely absent in patient no. A37. This latter patient also demonstrated a substantial reduction in a protein band that is believed to correspond to band 3, a glycoprotein anion transporter. This band was also diminished for patient no. A53.

Hemoglobin and Osmotic Fragility

Figure 2 shows typical osmotic fragility curves obtained from a LPL-deficient patient and a control subject. Both curves were of similar shape, and at physiologic osmotic force (~300 mOsm), the LPL-deficient patient exhibited an increased hemolysis. This was also reflected by the decreased hematocrit and increased plasma free hemoglobin observed in LPL deficiency (Table 3). At 300 mOsm, a 9.7% hemolysis was observed in LPL-deficient patients as compared with 1.1% in control subjects; decreasing osmotic force did not change the degree of hemolysis up to a force of 150 mOsm, a point at which hemolysis increased sharply to greater than 90% (Fig 2). When the osmotic fragility curves from all 21 controls and 23 LPL-deficient patients were pooled within each group and compared, the points of 50% hemolysis, as well as the slopes, were similar in both groups (Table 3). Correlation analyses showed that plasma free hemoglobin levels correlated positively with lysophosphatidylcholine levels ($r = .58$, $P = .0001$), whereas an inverse correlation was obtained with LDL cholesterol levels ($r = .40, P = .01$).

Table 4 shows the release of hemoglobin from hemolyzed erythrocytes after incubation in normolipidemic or LPL-deficient plasma. Hemolysis was increased when control erythrocytes were incubated with plasma from normolipidemic or LPL-deficient patients. However, the increase in plasma free hemoglobin was 13-fold greater when erythrocytes were incubated with LPL-deficient plasma. Correlation analysis showed a positive relation between hemoglobin concentrations before and after incubation in LPL-deficient plasma ($r = .61, P < .04$). Furthermore, incubation of control erythrocytes in chylomicron-free plasma obtained from LPL-deficient patients showed a significant increase in plasma free hemoglobin from 0.19 ± 0.14 to 0.31 ± 0.10 (mean ± SD, n = 10).

Echographic Measurements

Two LPL-deficient patients had been splenectomized before the study, the first one, 12 years before the study because of splenic hypertension and cysts, and the second, 9

Table 2. Chylomicron and Chylomicron-Free Plasma Phospholipid Composition in Primary LPL Deficiency

	Chylomicron	Chylomicron-Free		
	Patient (n = 22)	Control (n = 21)	Patient (n = 25)	*P*
Lysophosphatidylcholine	2.40 ± 1.67	6.35 ± 1.91	12.60 ± 5.82	.0001
Sphingomyelin	11.04 ± 1.06	16.30 ± 2.53	11.32 ± 7.08	.0038
Phosphatidylcholine	74.36 ± 12.22	71.04 ± 2.76	67.52 ± 12.37	NS
Phosphatidylinositol	2.33 ± 1.51	1.77 ± 0.39	2.39 ± 1.33	.0424
Phosphatidylserine	1.74 ± 2.18	0.43 ± 0.34	0.74 ± 0.54	.0258
Phosphatidylethanolamine	3.80 ± 2.33	2.76 ± 0.56	2.90 ± 1.60	NS

NOTE. Results are expressed as mole % (mean ± SD). NS, $P > .05$ *v* control.

years before the study due to splenomegaly and pancytopenia. In 12 patients, splenomegaly was documented by echography. Echographies of the remaining 12 patients showed normal spleens, and one had an accessory spleen. The 24 nonsplenectomized LPL-deficient patients were subdivided into two subgroups based on the presence or absence of splenomegaly. There were no significant differences in plasma free hemoglobin concentration, point of 50% hemolysis, or hemolysis at normal osmotic force (300 mOsm) between these two subgroups.

DISCUSSION

Plasma Free Hemoglobin

Control subjects had plasma free hemoglobin levels of 0.048 g/L, which is within normal limits (<0.06 g/L). In LPL-deficient patients, free hemoglobin was increased more than fivefold as compared with levels in control subjects (Table 3). In LPL-deficient patients, splenomegaly, elevated plasma free hemoglobin, and a lower hematocrit increase the possibility of erythrocyte destruction by hypersplenism.[20] Subdivision of these patients according to the presence or absence of splenomegaly did not reveal any difference for free hemoglobin or osmotic fragility between these two groups, suggesting that hypersplenism did not occur. Ferrans et al[21,22] have shown that splenomegaly in LPL deficiency could only be partially attributed to inflammatory mechanisms. The accumulation of ceroid bodies, as well as a sluggish erythrocyte transit, results in a congestion of the splenic red pulp, leading to splenomegaly. In LPL-deficient patients, haptoglobin and direct and indirect bilirubin levels were within normal limits, suggesting that hemolysis in LPL deficiency occurs due to erythrocyte susceptibility to mechanical stress.

Osmotic Fragility

Even though there was a slight tendency toward increased osmotic fragility in LPL deficiency, this increase did not reach statistical significance (Table 3). Instead, hemolysis was observed at high osmotic forces (Fig 2, Table 4) and did not change before the sharp break in the curve observed at 150 mOsm. Slopes of the hemolysis curves and the osmolarities at 50% hemolysis were not different between controls and LPL-deficient patients. These results suggest that erythrocytes from LPL-deficient patients were not more susceptible to hypo-osmotic lysis. In an earlier report on one LPL-deficient patient, Frohlich and Godin found an

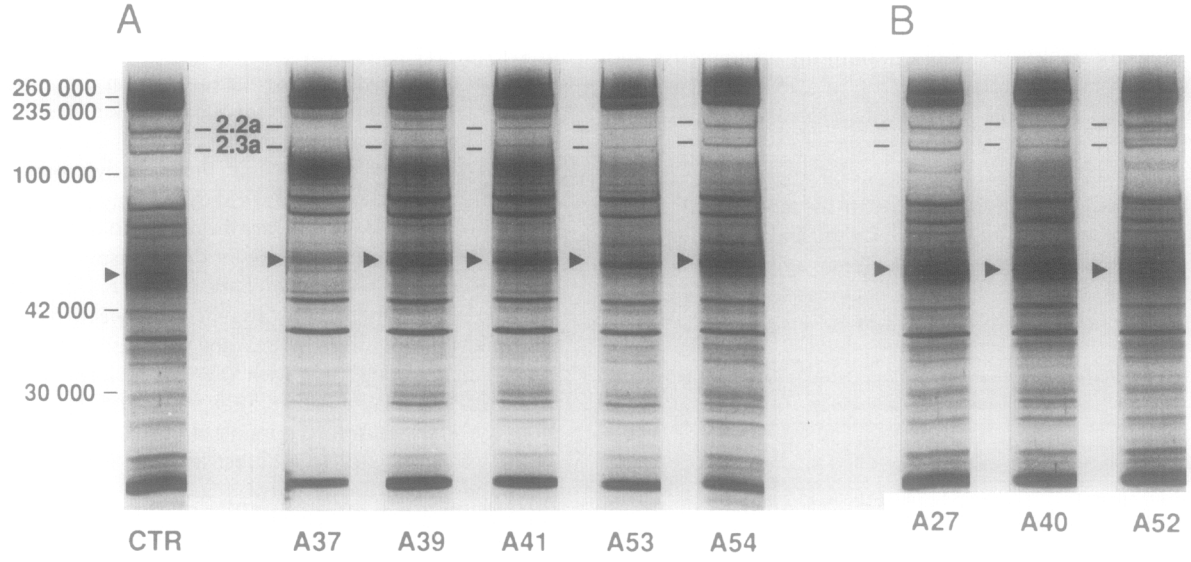

Fig 1. (A) Modifications in membrane protein composition in 5 patients suffering from LPL deficiency. Electrophoretic analysis revealed a considerable reduction of bands 2.2a and 2.3a (which appear as a doublet) in patients no. A37, A39, A41, A53, and A54. Note the absence of bands 2.2a and 2.3a in patient no. A37. Arrows indicate the putative band 3. (B) Electrophoretic analysis of erythrocyte ghost membrane proteins from LPL-deficient patients. Protein composition from patients no. A27, A40, and A52 appear normal as compared with the control (CTR).

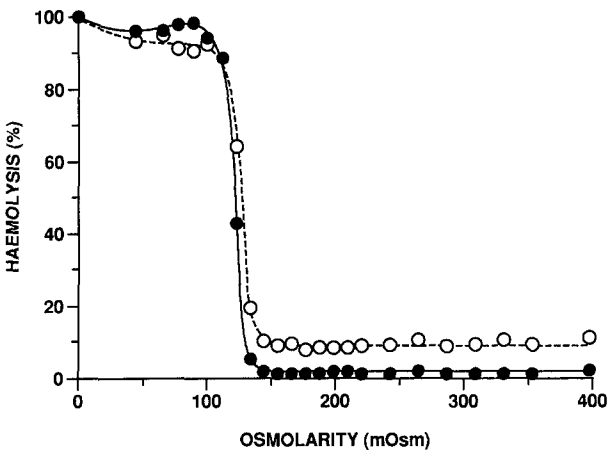

Fig 2. Typical osmotic fragility curves from a control subject (●) and a LPL-deficient patient (○).

increase in erythrocyte osmotic fragility.[4] This discrepancy between their results and ours may be attributed to a different experimental design and to the number of subjects studied.

Thus, data obtained from clinical observation and laboratory investigation suggest that hemolysis in LPL-deficient plasma occurs in vitro. Erythrocytes of control subjects hemolyze when incubated with plasma from LPL-deficient patients (Table 4). This in vitro hemolysis of control erythrocytes by LPL-deficient plasma suggests the presence of a hemolytic factor in these patients' plasma (Table 4). Furthermore, in vitro hemolysis is exacerbated in plasma obtained from patients with an already higher in vivo plasma free hemoglobin. Hemolysis of control erythrocytes by chylomicron-free plasma indicates that a hemolytic factor is present in the chylomicron-free plasma of LPL-deficient patients. However, the reduced hemolysis observed in the chylomicron-free fraction as compared with the whole chylomicronemic plasma could be due to the presence of hemolytic factors in both the chylomicron and chylomicron-free fractions and/or to a low initial plasma free hemoglobin in the isolated chylomicron-free plasma.

Protein Composition

It is conceivable that a defect in the erythrocyte membrane architecture is responsible for the leaking of hemoglobin and possibly other intracellular elements.[23-25] The membrane skeleton of the erythrocyte consists of a supramolecular complex of proteins, which serves to physically

Table 3. Plasma Free Hemoglobin and Osmotic Fragility Curves in Primary LPL Deficiency

	Control (n = 21)	LPL-Deficient (n = 26)	*P*
Hematocrit (%)	46 ± 2	39 ± 5	.0001
Plasma free hemoglobin (g/L)	0.048 ± 0.038	0.282 ± 0.331	.0025
Hemolysis at 300 mOsm (%)	1.1 ± 1.0	9.7 ± 8.6	.0001
Point of 50% hemolysis (mOsm)	121 ± 11	126 ± 6	NS
Slope of hemolysis curve	29.6 ± 5.5	26.8 ± 5.1	NS

NOTE. Results are the mean ± SD. NS, $P > .05$.

Table 4. Plasma Free Hemoglobin Measurements Before and After Incubation of Control Erythrocytes With Normal and LPL-Deficient Plasma

	Plasma Free Hemoglobin (g/L)		
	Before	After	Difference
Normolipidemic (n = 14)	0.030 ± 0.023	0.088 ± 0.059	0.058 ± 0.041
LPL-deficient (n = 12)	0.316 ± 0.050*	0.998 ± 0.215*	0.681 ± 0.192*

*$P < .0001$ *v* normolipidemic.

stabilize and confer the characteristic viscoelastic properties.[26] The principal components of the protein matrix are spectrin (α- and β-subunits, 260 and 235 kd, respectively), ankyrin (215 kd), band 3 (89 kd), actin (42 kd), glycophorin A (31 kd), and several other minor associated proteins.

Several lines of evidence demonstrate the importance of the cytoskeleton in maintaining the integrity of the erythrocyte membrane. The identification of a strain of anemic mice with a low spectrin content emphasizes this crucial role.[27] Numerous disorders of erythrocyte skeletal proteins have been associated with hemolytic anemia. In several cases of anemia, the primary erythrocyte defect is believed to be an abnormality in the spectrin-actin membrane skeleton.[24] Although hemolytic anemia has not been clinically detected in patients with LPL deficiency, the present study of cytoskeletal protein composition of eight randomly chosen patients was undertaken to identify possible qualitative and/or quantitative alterations with respect to membrane protein composition. Five LPL-deficient patients presented with abnormal concentrations of a high–molecular-weight protein doublet (2.2a and 2.3a). No obvious alterations were observed in the major cytoskeleton proteins such as spectrin and actin, although a thorough immunologic analysis will be required for definitive evaluation of any putative defects.

The role of the protein doublets 2.2a and 2.3a is currently unknown. However, since it is found in the membrane ghosts of erythrocytes, it may be involved in the anchorage of associated proteins to form the grid-like filament network responsible for the shape, strength, and deformability of the cell. The decrease in the putative band 3 of LPL-deficient patients no. A37 and A53 may result in more severe problems. Band 3, a membrane protein that contains an anion transporter,[28] possesses a cytoplasmic region that interacts with hemoglobin.[29] This domain is also linked to ankyrin, which in turn is attached to spectrin and the internal cytoskeleton.[30] Association of integral proteins like band 3 and glycophorin with the cytoskeleton would be expected to have a substantial effect on their lateral mobility.[30] It is therefore possible that this could be attributed, at least in part, to a defect in band 3. However, the two patients with this putative band 3 defect have almost normal plasma free hemoglobin levels. No relation has been found between these alterations and the type of LPL mutation present or the plasma lipid levels.

Alterations in the protein profiles of the membranes reported here could be mediated by calpain I, a cytosolic neutral calcium-dependent protease.[31] Hu and Bennett[32] have demonstrated that the cleavage of α-spectrin by

calpain I results in two polypeptides of 130 and 150 kd. However, in patients suffering from LPL deficiency, the level of α-spectrin was normal and no such polypeptide fragments were observed.

Plasma and Phospholipid Composition

LPL-deficient patients had higher plasma phospholipid concentrations, characterized by an accumulation of lysophosphatidylcholine. Considering the low levels of lysophosphatidylcholine in erythrocyte membranes, it is unlikely that the increase in plasma lysophosphatidylcholine concentrations could be explained by the release of erythrocyte lysophospholipids. The increase in lysophosphatidylcholine concentration coupled with the decrease in phosphatidylcholine concentration suggests that a conversion of phosphatidylcholine to lysophosphatidylcholine may account for a part of the accumulated lysophosphatidylcholine. LCAT,[33] phospholipase A,[33] and to some extent hepatic lipase[34] are responsible for the normal conversion of phosphatidylcholine to lysophosphatidylcholine. However, LCAT requires high-density lipoprotein to produce this activity,[33] and since LPL-deficient patients are hypoalphalipoproteinemic and have increased free cholesterol levels (Table 1), LCAT activity is probably reduced and cannot account for the accumulation of lysophosphatidylcholine. Moreover, hepatic lipase activity is normal, and the preferred substrates for the phospholipases are phosphatidylethanolamine and phosphatidylglycerol,[35] both of which are found in minimal concentrations. It therefore appears likely that the accumulation of lysophosphatidylcholine is due to an impairment in the reverse metabolic pathway converting lysophosphatidylcholine back to phosphatidylcholine. This activity has been termed lysolecithin acyltransferase and is catalyzed under isoenergetic conditions in the presence of low-density lipoprotein by LCAT.[33] The accumulation of lysophosphatidylcholine in LPL deficiency is probably the result of the underlying hypobetalipoproteinemia (Table 1), which breaks the equilibrium between LCAT and lysolecithin acyltransferase activities.

The Role of Lysophosphatidylcholine

The most striking feature of LPL-deficient plasma is the accumulation of lysophosphatidylcholine. It has been shown that lysophosphatidylcholine can rapidly be taken up when it comes in contact with erythrocyte membranes,[36] thereby increasing membrane lysophosphatidylcholine concentration. This enrichment in lysophosphatidylcholine could then lead to morphologic changes of the erythrocyte.[36,37] However, in LPL deficiency, membrane lysophosphatidylcholine concentration is decreased,[11] thus excluding the lysolecithin effect on complement-mediated lysis.[38] However, the fatty acid composition of these lysolipids could play a more important role than the variations in phospholipids per se. It has been established in vitro that plasma fatty acid concentration and composition is an important determinant of erythrocyte stability.[36,39-46] The only fatty acid data available for LPL deficiency have described an abnormal plasma fatty acid composition characterized mainly by a decrease in linoleic acid.[47]

This study represents the investigation of a unique in vivo model in which a significant lysophosphatidylcholine accumulation coexists with an increase in plasma free hemoglobin. The observed hemolysis is caused by factors present in plasma of LPL-deficient patients. Erythrocyte osmotic fragility of LPL-deficient patients was not different from that of control subjects, but lysophosphatidylcholine has been shown to protect against lysis under conditions of rapid swelling.[48] Since erythrocyte fragility measurements were made under rapid-swelling conditions, it is possible that an increased susceptibility has not been detected. These findings suggest that the observed hemolysis could be due to changes in the lipid composition of the erythrocyte membrane.

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