

PREMATURE ATHEROSCLEROSIS IN PATIENTS WITH FAMILIAL CHYLOMICRONEMIA CAUSED BY MUTATIONS IN THE LIPOPROTEIN LIPASE GENE

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ABSTRACT

Background Patients with lipoprotein lipase deficiency usually present with chylomicronemia in childhood. The syndrome has been considered non-atherogenic primarily because of the low levels of low-density lipoprotein (LDL) cholesterol. We prospectively evaluated patients with lipoprotein lipase deficiency for atherosclerosis.

Methods Evidence of carotid, peripheral, and coronary atherosclerosis was sought in four patients (two men and two women) with the phenotype of familial chylomicronemia by clinical examination over a period of 14 to 30 years and by Doppler ultrasonography, ultrasonography, and exercise-tolerance testing after the age of 40. Angiography was performed when indicated. Lipoprotein lipase deficiency was assessed in vivo and in vitro by functional assays and DNA-sequence analysis.

Results All four patients had a profound functional deficiency of lipoprotein lipase with a reduced enzymatic mass due to missense mutations on both alleles of the lipoprotein lipase gene. In all four patients, peripheral or coronary atherosclerosis (or both) was observed before the age of 55. Despite following a low-fat diet in which fat composed 10 to 15 percent of the daily caloric intake, the patients had hypertriglyceridemia (mean \pm SD triglyceride level, 2621 ± 1112 mg per deciliter [29.59 ± 12.55 mmol per liter]), low plasma levels of high-density lipoprotein cholesterol (17 ± 7 mg per deciliter [0.43 ± 0.18 mmol per liter]), and very low levels of LDL cholesterol (28 ± 16 mg per deciliter [0.72 ± 0.41 mmol per liter]). Three patients had one risk factor for atherosclerosis, whereas in one male patient, heavy smoking and diabetes were associated with an accelerated course of the disease.

Conclusions Premature atherosclerosis can occur in patients with familial chylomicronemia as a result of mutations in the lipoprotein lipase gene. Defective lipolysis may increase susceptibility to atherosclerosis in humans. (N Engl J Med 1996;335:848-54.)

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THE role of triglycerides in atherosclerosis¹ is controversial. We investigated the role of triglyceride-rich particles in atherosclerosis by studying patients with an inherited disorder of triglyceride metabolism, deficiency of lipoprotein lipase.

Lipoprotein lipase is the rate-limiting enzyme for

the hydrolysis and removal of chylomicrons and very-low-density lipoprotein (VLDL) triglycerides from the circulation.² This enzyme is a dimer that acts at the endothelial surface of extrahepatic capillaries, providing cells with fatty acids for either energy or storage. Lipolysis also initiates a cascade of conversion of lipoprotein particles, which results in circulating low-density lipoprotein (LDL) and in the remodeling of high-density lipoprotein (HDL). In addition, lipoprotein lipase enhances the binding of non-high-density lipoproteins to the extracellular matrix and the uptake of these proteins by cell-specific receptors through mechanisms independent of lipolysis.³

Patients with two defective alleles for the lipoprotein lipase (*LPL*) gene or its cofactor, apolipoprotein C-II, usually present early in life with recurrent abdominal pain, eruptive xanthomatosis, lipemia retinalis, hepatosplenomegaly, and chylomicronemia, often complicated by acute pancreatitis, which can be prevented by a low-fat diet.⁴ Over 60 mutations of the *LPL* gene can cause lipoprotein lipase deficiency.⁵ Interestingly, 3 to 7 percent of whites are heterozygous carriers⁶⁻⁹ and may have altered lipoprotein phenotypes that predict an increased risk of atherosclerosis.

Earlier reports suggested that lipoprotein lipase deficiency does not predispose patients to atherosclerosis.¹⁰⁻¹² Younger patients with familial chylomicronemia do not show signs of cardiovascular disease, and preliminary autopsy studies revealed no serious atherosclerotic lesions.^{13,14} The concept that atherosclerosis was not a feature of lipoprotein lipase deficiency came to be accepted because chylomicrons were thought to be too large to penetrate the endothelial barrier and because of the abnormally low levels of circulating LDL cholesterol and other proatherogenic particles in these patients. In addition, these patients are not obese and generally follow a low-fat diet.

Over a period of two to three decades, we pro-

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spectively evaluated four adults with familial chylomicronemia caused by defined mutations in the *LPL* gene and found atherosclerosis involving both coronary and peripheral vessels.

METHODS

Patients

We studied two men and two women with chylomicronemia and a history of abdominal pain. The patients were studied for an average of 22 years (range, 14 to 30).

Biochemical Analyses

Plasma lipid profiles were determined once or twice a year; all the patients were consuming a low-fat diet in which fat represented 10 to 15 percent of the daily caloric intake. Fasting plasma cholesterol and triglyceride concentrations were determined enzymatically.^{15,16} HDL cholesterol was measured by heparin-manganese precipitation.¹⁷ Plasma LDL cholesterol (density, 1.006 to 1.063 g per milliliter) was measured by sequential ultracentrifugation.¹⁸ Lipoprotein lipase and hepatic lipase activities were measured in plasma by a radiolabeled glycerol tri[³H]oleate emulsion¹⁹ after the intravenous injection of 50 IU of heparin per kilogram of body weight. The mass of lipoprotein lipase in untreated and heparin-treated plasma samples was measured by enzyme-linked immunosorbent assay (ELISA) with the monoclonal antibodies 5D2 and 5F9.²⁰ The presence of plasma apolipoprotein C-II was determined by isoelectric focusing. ELISAs were used to measure plasma lipoprotein(a) (Behring) and plasminogen-activator inhibitor type 1 (Stago Diagnostics). Plasma factor VII was measured with an assay according to the manufacturer's recommendations (Behring), and a radioenzymatic assay was used to test for homocysteinemia.²¹

Molecular Analyses

All the exons of the *LPL* gene were analyzed by amplification with the polymerase chain reaction (PCR) and single-strand conformation polymorphisms of genomic DNA.²² When a gene variant was detected, the corresponding PCR product was sequenced directly.²³ The functional effects of a newly identified missense

mutation were tested in vitro by site-directed mutagenesis in COS-1 cells as described previously.²⁴ Three separate transfections were performed with mutant and wild-type clones. The mass and activity of lipoprotein lipase were determined in cell-culture medium.^{19,20} Genotyping of apolipoprotein E was performed.²⁵

RESULTS

Patient 1

A 54-year-old woman who since childhood had had episodes of abdominal pain induced by a high intake of fat was 1.58 m tall, weighed 51.8 kg, and had a body-mass index (the weight in kilograms divided by the square of the height in meters) of 21.6 (Fig. 1). At the age of 24, when she was 10 weeks pregnant, chylomicronemia (plasma triglyceride level, 5900 mg per deciliter [66.61 mmol per liter]) was discovered, when she presented with eruptive xanthomata and hepatomegaly. After the institution of an extremely-low-fat diet (in which fat composed less than 10 percent of total calories), her triglyceride levels decreased to below 1000 mg per deciliter (11.29 mmol per liter) and her cholesterol levels decreased to below 400 mg per deciliter (10.3 mmol per liter). The pregnancy and delivery were otherwise uneventful. Three years later, the patient had another, uncomplicated pregnancy and delivery (maximal levels at 30 weeks of gestation: triglycerides, 3150 mg per deciliter [35.56 mmol per liter]; total cholesterol, 675 mg per deciliter [17.5 mmol per liter]). She continued to follow a strict dietary regimen, did not take estrogen therapy, and remained free of pancreatitis. She was a compound heterozygote for two known mutations of the *LPL* gene (Gly188Glu²³ and Arg243Cys²⁶), which resulted in extremely low plasma lipoprotein lipase activity (Table 1).

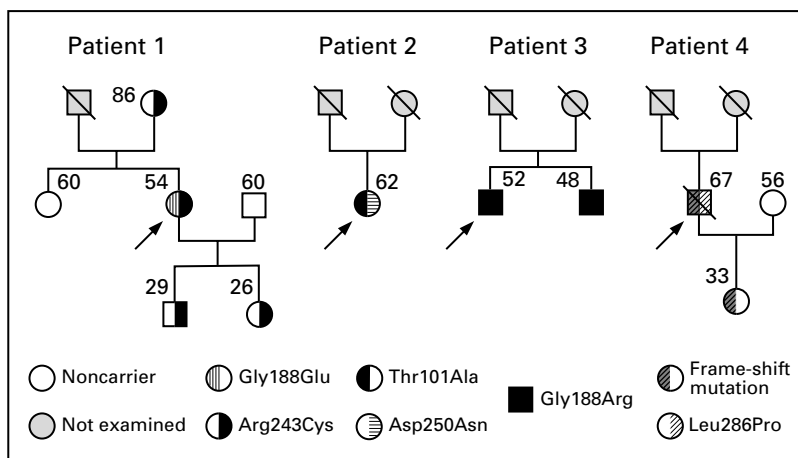


Figure 1. Pedigrees of the Four Patients with Lipoprotein Lipase Deficiency and Mutations of Both Alleles of the *LPL* Gene.

Arrows indicate the probands. Numbers indicate ages. Squares denote male family members, circles female family members, and symbols with a slash deceased family members.

Annual assessments revealed increasing levels of plasma total cholesterol during fasting, together with hypertriglyceridemia (Table 2). Ciprofibrate therapy (100 mg per day) had no effect. The patient had smoked five cigarettes a day for 20 years. Her father, who had combined hyperlipidemia, first had angina at the age of 63 and died 15 years later of a myocardial infarction. Her mother, who was 86, had had a probable transient ischemic attack at the age of 75 and a myocardial infarction at the age of 85.

At the age of 45 the patient underwent combined Doppler ultrasonography and ultrasonography, which revealed no abnormalities of the carotid or peripheral arteries. Angina was evident when the patient was 51 years old, despite the fact that she had a normal exercise-tolerance test. Angiography revealed single-vessel disease of the right coronary artery (90 percent stenosis of the second segment) without any spasm or myocardial impairment, which was treated with percutaneous transluminal coronary angioplasty. When the patient was 54, arterial thickening of the carotid and vertebral arteries as well as of the iliofemoral arteries was detected (Table 3).

Patient 2

A 62-year-old woman who was 1.58 m tall, weighed 48 kg, and had a body-mass index of 20.8 had had episodes of abdominal pain from childhood that were associated with chylomicronemia (Fig. 1). The patient was first examined at the age of 39 after an episode of acute pancreatitis (triglyceride level, 3200

mg per deciliter [36.13 mmol per liter]). She followed a strict low-fat diet, did not take estrogen, and remained free of pancreatitis. Lipoprotein lipase activity was very low in heparin-treated plasma (Table 1) and was associated with low lipoprotein lipase mass and an absence of lipoprotein lipase dimers. Patient 2 was a compound heterozygote for one novel mutation (Thr101Ala) and a previously described mutation (Asp250Asn).²⁷ The Thr101Ala mutation disrupted a conserved residue²⁸ and was associated with low lipoprotein lipase mass and activity in vitro.

Over the course of two decades, this patient's lipid profiles revealed increasing cholesterol and triglyceride levels (Table 2). Mild glucose intolerance was detected when she was 51 and was treated with glyburide (7.5 mg daily). Therapy with gemfibrozil (900 to 1350 mg per day) was initiated when the patient was 52, with a poor response. Her mother had died at the age of 80 of a myocardial infarction.

At the ages of 49 and 51 the patient underwent arterial Doppler ultrasonography, which revealed no abnormalities. A fibromatous atherosclerotic plaque (diameter, 3 mm) at the origin of the left internal carotid artery was identified by ultrasonography when the patient was 52 (Table 3). At this time the patient also began to report having angina pectoris, which became unstable four years later. This finding led to coronary angiography, which revealed subtotal thrombosis of the right coronary artery (without any ventricular dysfunction) and 90 percent stenosis of the first diagonal branch of the left anterior de-

TABLE 1. FUNCTIONAL AND MOLECULAR CHARACTERISTICS OF LIPOPROTEIN LIPASE DEFICIENCY IN FOUR PATIENTS.*

CHARACTERISTIC	NORMAL VALUE†	PATIENT 1	PATIENT 2		PATIENT 3			PATIENT 4			
			IN VIVO‡		IN VITRO‡		IN VIVO‡		IN VITRO‡		
			Normal Allele	Mutated Allele	Normal Allele	Mutated Allele	Normal Allele	Mutated Allele	Normal Allele	Mutated Allele	
LPL											
Mass (ng/liter)	325±101	146.8	86.4	1085±247	626±168	237.2	531±81	376±121	107.1	531±81	534±103
Monomers (ng/liter)	84±44	98.3	86.4	450±56	378±78	170.3	214±47	281±93	83.2	214±47	420±94
Dimers (ng/liter)	241±58	48.5	ND	635±191	248±166	67	287±25	95±29	23.9	287±25	114±9
Dimer:monomer	2.85±1.6	0.49	ND	1.5±0.5	0.6±0.4	0.39	1.4±0.2	0.34±0.01	0.28	1.4±0.2	0.27±0.04
Activity (mU/ml)	212±50	25	2	59.4±5.4	16.4±1.7	17	92±5	0.53±0.5	14	92±5	0.75±0.4
Apolipoprotein C-II		+	+			+			+		
Hepatic lipase activity (mU/ml)	413±91	143	311			252			215		
LPL gene mutations		Gly188Glu (GGG→GAG in exon 5) Arg243Cys (CGC→TGC in exon 6)		Thr101Ala (ACC→GCC in exon 3)§¶ Asp250Asn (GAC→AAC in exon 6)		Homozygous for Gly188Arg (GGG→AGG in exon 5)§¶			Frame shift→Stop119 (ΔCCGCVGG in exon 3)§ Leu286Pro (CTG→CCG in exon 6)§¶		

*Plus-minus values are means ±SD. ND denotes not detectable, and a plus sign the presence of the protein.

†Values were determined in heparin-treated plasma.

‡The values are the means ±SD of three measurements in cell-culture medium and are expressed as nanomoles of free fatty acid per minute per milliliter.

§This is a novel mutation.

¶The effects of the mutation were assessed in vitro.

TABLE 2. PLASMA LIPID AND LIPOPROTEIN LEVELS AND RISK FACTORS FOR ATHEROSCLEROSIS IN THE FOUR PATIENTS.*

VARIABLE	PATIENT 1	PATIENT 2	PATIENT 3	PATIENT 4
Plasma lipids and lipoproteins — mg/dl (no. of measurements)†				
Total cholesterol				
Mean ±SD	293±120 (33)	200±60 (20)	280±79 (14)	422±223 (38)
Range	100–528	110–340	190–460	120–1050
Triglycerides				
Mean ±SD	2027±140 (33)	1830±650 (20)	2036±960 (14)	4590±2700 (38)
Range	220–5900	340–3360	600–3800	500–12,800
HDL cholesterol				
Mean ±SD	16±8 (15)	16±6 (20)	12, 24 (2)	16±10 (35)
Range	5–38	9–30		8–56
LDL cholesterol‡	27	15	18	51
Risk factors for atherosclerosis				
Sex	F	F	M	M
Family history of atherosclerosis	Yes	Yes	Yes	Yes
Body-mass index	21.6	20.8	19.2	24.3
Diabetes	No	Glucose intolerance§	No	NIDDM, IDDM at age 54
Hypertension	No	No	No	Transient§
Cigarette use	5/day for 20 yr	No	15/day for 20 yr	40–50/day for 45 yr
Alcohol use	No	No	10–20 g/day	60–130 g/day
Apolipoprotein E isoforms	E3/E3	E3/E3	E3/E3	E3/E3
Lipoprotein(a) — g/liter¶	0.05	0.01	—	0.10
Fibrinogen — g/liter	3.5	2.6	5.0	4.3
Plasma levels of PAI-1, factor VII, homocysteine	Normal	Normal	—	—

*NIDDM denotes non-insulin-dependent diabetes mellitus, IDDM insulin-dependent diabetes mellitus, and PAI-1 plasminogen-activator inhibitor type 1.

†To convert values for cholesterol to millimoles per liter, multiply by 0.02586; to convert values for triglycerides to millimoles per liter, multiply by 0.01129.

‡The normal value is 140±25 mg per deciliter (3.62±0.65 mmol per liter).

§This condition was well controlled by appropriate therapy.

¶The normal value is <0.30 g per liter.

||This value was not determined during an acute episode. The normal value is <4.5 g per liter.

scending coronary artery. New fibroatheromatous plaques were later observed at both carotid bifurcations and in the subclavicular and vertebral arteries. In addition, there was progression of previously reported lesions. Diffuse arterial endarteritis along the abdominal aorta and new fibroatheromatous plaques were detected along the common femoral, the superficial femoral, and the popliteal arteries. No calcification was reported.

Patient 3

A 52-year-old man who was 1.78 m tall, weighed 61.8 kg, and had a body-mass index of 19.2 was the older of two brothers with familial chylomicronemia (Fig. 1). The disease was diagnosed when he was 29 years old (triglyceride, 3800 mg per deciliter [42.9 mmol per liter]), after the disorder was identified in a first cousin. The patient had reported abdominal pain induced by a high intake of fat but had never had acute pancreatitis. He followed a low-fat diet and had a moderate intake of alcohol. He was homozygous for a novel mutation (Gly188Arg) in exon 5 of the *LPL* gene, and he had a low lipopro-

tein lipase mass and very low lipoprotein lipase activity in vivo and in vitro (Table 1).

He had smoked 15 cigarettes a day for 20 years (Table 2). His parents had had normal blood lipid levels. At the age of 49, he underwent Doppler ultrasonography and ultrasonography, which showed several irregular and calcified atherosclerotic plaques together with a large ulcerative calcified plaque projecting into the lumen (causing luminal narrowing of 30 to 40 percent) at the bifurcation of the right carotid artery and two calcified and ulcerated plaques (causing luminal narrowing of 30 percent) on the left. Arterial thickening was observed in the inferior limbs with no organized plaque (Table 3). The patient was free of angina, and his results on exercise-tolerance tests were consistently normal.

Patient 4

A 67-year-old man with a history of abdominal pain induced by a high intake of fat and hyperlipidemia was 1.61 m tall, weighed 74 kg, and had a body-mass index of 24.3. When he was 53, during an episode of acute pancreatitis, eruptive xanthomata and

TABLE 3. SIGNS AND SYMPTOMS OF ATHEROSCLEROSIS IN THE FOUR PATIENTS.*

SIGN OR SYMPTOM	PATIENT 1	PATIENT 2	PATIENT 3		PATIENT 4
			age (yr) at onset (finding)		
Cervical atherosclerosis					
Common carotid artery	54 (infiltration)	ND	ND		53 (plaque), 64 (30% stenosis)
Carotid bifurcation	54 (infiltration)	60 (plaque)	49 (plaque; narrowing, 30–40%)		60 (plaque), 64 (50% stenosis)
Internal carotid artery	ND	52 (plaque)	49 (plaque; narrowing, 30%)		53 (plaque), 56 (40% stenosis)
Subclavian artery	ND	60	ND		63 (plaque)
Vertebral artery	54 (infiltration)	60	ND		53 (plaque)
Coronary arterial disease					
Angina	51	52	ND		57
Ischemic event	ND	56 (unstable angina)	ND		65 (myocardial infarction), 67 (sudden death)
Coronary lesions	51 (90% stenosis of RCA)	56 (thrombosis of RCA; 90% stenosis of Dg1)	ND		65 (thrombosis of RCA; 90% stenosis of Mg1; diffuse arteriosclerosis)
Peripheral arterial disease					
Clinical signs	ND	ND	ND		54 (weak pulse)
Claudication	ND	ND	ND		58
Ischemic event	ND	ND	ND		65 (subacute ischemia of the inferior limbs)
Abdominal aorta	ND	59 (plaque)	ND		65 (plaque)
Iliac artery	54 (infiltration)	60 (plaque)	49 (infiltration)		53 (plaque), 58 (40% stenosis), 65 (90% stenosis)
Common femoral artery	54 (infiltration)	59 (plaque)	49 (infiltration)		55 (bruit), 59 (30% stenosis), 62 (80% stenosis)
Superficial femoral artery	ND	60 (plaque)	ND		58 (40% stenosis), 60 (70% stenosis), 62 (thrombosis)

*In each case of stenosis the percentage of luminal narrowing is for the most advanced lesion. ND denotes not detected, RCA right coronary artery, Mg1 first marginal branch of the circumflex coronary artery, and Dg1 first diagonal branch of the left anterior descending coronary artery.

chylomicronemia (triglyceride level, 12,800 mg per deciliter [144.50 mmol per liter]) were noted. Ciprofibrate therapy (100 mg per day) was ineffective. He had a second episode of acute pancreatitis at the age of 58. Plasma lipoprotein lipase activity was extremely low (Table 1), and the level of lipoprotein lipase dimers was also low. The patient was a compound heterozygote for two novel mutations of the *LPL* gene: a Leu286Pro mutation and a complex gene rearrangement (a deletion of four nucleotides and an insertion of two nucleotides at position 290 of the complementary DNA) leading to a frameshift mutation and a stop codon at residue 119. The Leu286Pro mutation modified a conserved residue²⁸ and resulted in nearly undetectable lipoprotein lipase activity in vitro.

Glucose intolerance was present when the patient was 53 years of age (Table 2). He required insulin therapy at the age of 54 and had overt exocrine pancreatic insufficiency one year later. Despite poor control of diabetes, renal and retinal complications were absent. He smoked 40 to 50 cigarettes per day and drank at least 60 g of alcohol per day. His mother, who was obese and had hypertension, had died of a stroke at the age of 73 years.

His anterior tibial pulses were decreased at the age of 53 (Table 3). Ultrasonography revealed calcified atherosclerotic plaques of the cervical and iliac arter-

ies. The patient first reported having angina pectoris at the age of 57 and intermittent claudication at the age of 58. At the age of 65, he underwent aortofemoral bypass graft surgery, during which atherosclerotic lesions of all grades (from fatty streaks to calcified and hemorrhagic atherosclerotic plaques) were noted all along the aorta and iliac and femoral arteries. At that time, coronary angiography revealed a prior inferior myocardial infarction associated with a thrombus of the right coronary artery, as well as diffuse arterial lesions inaccessible to any surgical procedure. The patient died suddenly two years later at the age of 67.

DISCUSSION

In these four patients with familial lipoprotein lipase deficiency resulting in chylomicronemia, there were signs and symptoms of atherosclerosis before the age of 55. In a previously described 75-year-old patient with lipoprotein lipase deficiency,²⁹ coronary heart disease and signs of peripheral atherosclerosis were also evident. Therefore, in these patients, lipoprotein lipase deficiency did not provide complete protection against atherosclerosis.

Our findings appear to contradict previous reports suggesting that atherosclerosis is unlikely to develop in patients with lipoprotein lipase deficiency,^{4,10-14} supposedly because these patients have profoundly

reduced levels of circulating remnant lipoproteins³⁰⁻³³ and very low levels of LDL cholesterol.³³ These patients usually also have low body-mass indexes and follow a low-fat diet — both of which are associated with a reduced risk of atherosclerosis.

Therefore, other metabolic disturbances must be invoked to account for our findings. These patients have high levels of triglycerides and low levels of HDL cholesterol.^{1,34} In addition, postprandial clearance of triglyceride-rich particles is severely delayed, which may expose lipoproteins to oxidation.³⁵ Moreover, reverse cholesterol transport may be impaired as a result of an alteration in the composition of HDL particles, which are cleared more rapidly from the circulation.^{36,37} Therefore, these patients have a lipoprotein profile that is reminiscent of the profile in the postprandial state during which atherogenic remnant particles are produced and the numbers of antiatherogenic particles (HDL) are decreased.

Furthermore, turnover studies have shown that VLDL is normally converted into intermediate-density lipoprotein and LDL,³² presumably through hepatic lipase activity,³⁴ and LDL may increase in patients who are following a low-fat (but carbohydrate-enriched) diet.^{10,31} However, the LDL cholesterol levels in our patients never rose above 60 mg per deciliter (1.55 mmol per liter) — levels usually associated with protection against atherosclerosis.

Other evidence suggests that the relation between lipoprotein lipase and atherogenesis is not mediated solely by changes in plasma lipoproteins. Lipoprotein lipase has been proposed as one of the key proteins involved in the retention of LDL and VLDL in the arterial intima, by enhancing their adherence to the extracellular matrix.³⁸ Moreover, local secretion of lipoprotein lipase by macrophages may enhance the uptake of atherogenic lipoproteins, thereby increasing the formation of foam cells.³ These functions may be independent of the catalytic activity of lipoprotein lipase. All four of our patients had missense mutations that profoundly impaired lipolysis but preserved the mass of lipoprotein lipase. Therefore, such mutations may be proatherogenic by promoting an altered lipoprotein profile while favoring lipoprotein retention and foam-cell formation in the arterial wall.

Mutations of the *LPL* gene that impair catalytic activity but have no effect on the mass of the enzyme are particularly common in whites, affecting 3 to 7 percent.⁶⁻⁸ Persons who are heterozygous for these mutations may have higher fasting triglyceride levels and lower HDL cholesterol levels.^{6,7} These lipoprotein abnormalities may increase the risk of cardiovascular disease¹ and are associated with enhanced progression of moderate coronary lesions.³⁹ Moreover, heterozygotes have an altered response to a dietary fat challenge.⁴⁰ Our results provide *in vivo* evidence that atherosclerosis may develop in patients with fa-

miliar chylomicronemia as a result of lipoprotein lipase deficiency and suggest that normally functioning lipoprotein lipase in plasma may confer protection against atherosclerosis in humans.

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CORRECTION

Premature Atherosclerosis in Patients with Familial Chylomicronemia Caused by Mutations in the Lipoprotein Lipase Gene

Premature Atherosclerosis in Patients with Familial Chylomicronemia Caused by Mutations in the Lipoprotein Lipase Gene . In the Abstract and on pages 850, 851, and 852, when the word "ultrasonography" is used alone, it should read, "B-mode ultrasonography."