Alterations in plasma lipoproteins and apolipoproteins before the age of 40 in heterozygotes for lipoprotein lipase deficiency

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Abstract We have assessed the expression of heterozygosity for lipoprotein lipase (LPL) deficiency by studying a single large French Canadian family comprising 92 persons including 21 carriers of the catalytically defective P207L mutation. Phenotypic changes distinguishing heterozygotes from controls were seen early, before the age of 40 and often before 20 years of age. In the total cohort these changes included an elevation in the mean very low density (VLDL) and intermediate density lipoprotein (IDL) triglyceride (+69%; P = 0.01 and +40%; P = 0.001) and cholesterol (+51%; P = 0.03 and +67%; P = 0.007) and apoB levels but decreased HDL_2 and HDL_3 cholesterol, (-32%; P = 0.01 and -15%; P = 0.002 respectively). While the lipid compositions of VLDL and IDL were similar between heterozygotes and controls, the low density (LDL) and high density lipoproteins (HDL) of carriers were triglyceride enriched. Heterozygotes also had a markedly lower apoC-III ratio (apoC-III in supernatant/apoC-III in heparin precipitate) (1.46 vs. 3.86 $P = 1 \times 10^{-4}$) indicating a substantial enrichment of VLDL and IDL with apoC-III and depletion of HDL apoC-III supporting this ratio as an effective index for efficiency of lipolysis. LpA-I was markedly reduced (0.34 vs. 0.43 $P = 1 \times 10^{-5}$) showing that levels of this particle are partly dependent on LPL catalytic activity. Heterozygotes manifest from an early age with a markedly reduced HDL, LpA-I, apoC-III ratio and an increased TC/HDLc ratio which would predict a relatively increased risk of premature coronary artery disease, compared to their normal siblings.—**Bijvoet, S., S. E. Gagné, S. Moorjani, C. Gagné, H. E. Henderson, J-C. Fruchart, J. Dallongeville, P. Alaupovic, M. Prins, J. J. P. Kastelein, and M. R. Hayden.** Alterations in plasma lipoproteins and apolipoproteins before the age of 40 in heterozygotes for lipoprotein lipase deficiency. *J. Lipid Res.* 1996. **37:** 640–650.

Supplementary key words lipoproteins • heterozygotes • lipoprotein lipase • LPL deficiency

Lipoprotein lipase (LPL EC3.1.1.34) is a multi-functional protein. After synthesis in parenchymal cells, primarily adipose tissue and skeletal muscle, it is transported to the intimal surface of the vascular endothelium where it is anchored by the heparan sulphate side chains of membrane proteoglycans and plays a pivotal role in the hydrolysis of lipoprotein triacylglycerols to monoacylglycerols and fatty acids (1). LPL has recently also been shown to have other important metabolic functions where it acts as a ligand for the low density lipoprotein receptor related protein (LRP) (2) and influences the secretion and uptake of low density lipoprotein cholesterol (3–6).

Chylomicronemia, in association with LPL deficiency, was the phenotype originally chosen to search for naturally occurring mutations in the LPL gene. Patients with this phenotype had severe clinical sequelae and often presented in infancy with abdominal pain, pancreatitis, hepatosplenomegaly, and failure to thrive (7–9). More recently however, it has been clearly established that mutations on both alleles of the LPL gene may, more frequently, be associated with partial LPL activity and a

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Abbreviations: LPL, lipoprotein lipase; apo, apolipoprotein; LRP, lipoprotein receptor-related protein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; apoC-III-W, apoC-III measured in whole serum; apoC-III-HP, apoC-III measured in heparin-precipitated lipoproteins; apoC-III-HS, apoC-III measured in heparin supernate lipoproteins; CE, cholesteryl ester; UC, unesterified cholesterol; PL, phospholipid.

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milder clinical phenotype. For these patients the lipoprotein abnormalities may be varied (10). We have shown for example, that a mutation in the human LPL gene, $Asn^{291} \rightarrow {}^{Ser}$ (N291S), which results in a partial deficiency of lipolytic activity, is present with increased frequency in patients with certain lipid disorders, in particular, familial dysbetalipoproteinemia (11) and familial combined hyperlipidemia (12) and may also be associated with hypoalphalipoproteinemia in patients with coronary artery disease (13).

However, the natural history and biochemical consequence of heterozygosity for mutations in the LPL gene remains uncertain. Two prior studies have attempted to explore this question in individual kindreds with DNA verified cohorts of carriers. One of these, examined members of an extended pedigree, with the $Gly^{188} \rightarrow {}^{Glu}$ mutation (G188E), and identified age as an environmental factor that modulated the lipoprotein phenotype of carriers (14). Hypertriglyceridemia was shown to be strongly age dependent with differences in triglyceride levels between carriers and non-carriers only observed after the age of 40 years.

Another study of two smaller kindreds, heterozygous for the same G188E mutation, demonstrated that although carriers showed no marked differences in fasting triglyceride levels their post-prandial lipemia was prolonged (15). Impairment in lipolysis was evident after a fat challenge and was manifest in carriers with significant disturbances in lipoprotein levels and composition. However, detailed comparisons of carriers and non-carriers in these different families was not feasible due to the small number of carriers (n = 8) and controls (n = 8) with varied age (mean = 49.5 yr for carriers and 27.3 for controls) and sex ratios. Furthermore, this study was also potentially confounded by other genetic factors as the different families analyzed were not related.

We have previously shown that LPL gene mutations resulting in a catalytically inactive lipase are present at a high frequency in French Canadians of the Province of Quebec. In this population the $Pro^{207} \rightarrow {}^{Leu}$ mutant allele (P207L) is the most prominent, accounting for 73% of the mutant alleles in homozygotes (16, 17) while two other mutations, G188E (18) and $Asp^{250} \rightarrow {}^{Asn}$ substitutions (D250N) (19), are found in the remainder. The high frequency of LPL deficiency in Quebec, associated with relative isolation in certain rural areas and large families, has provided unique opportunities for assessment of the phenotypic expression of heterozygotes for LPL deficiency.

In this study we have examined members of a single large extended family in which carriers of the P207L mutation appear in four generations. We have recorded data for members below the age of 40 years only as only one carrier was older. We show that heterozygotes for the P207L mutation manifest with significant differences in lipoprotein lipid and protein composition, often before the age of 20.

This study clearly demonstrates the importance of LPL in determining the concentration of plasma lipoproteins and the composition of LDL and HDL lipids and apolipoproteins. It also provides additional in vivo evidence of the catalytic and other roles of LPL in neutral lipid transport. Furthermore, we show that carriers manifest with an increased VLDL + IDL apolipoprotein C-III (apoC-III) content and a decreased HDL apoC-III component supporting the role of lipolysis in

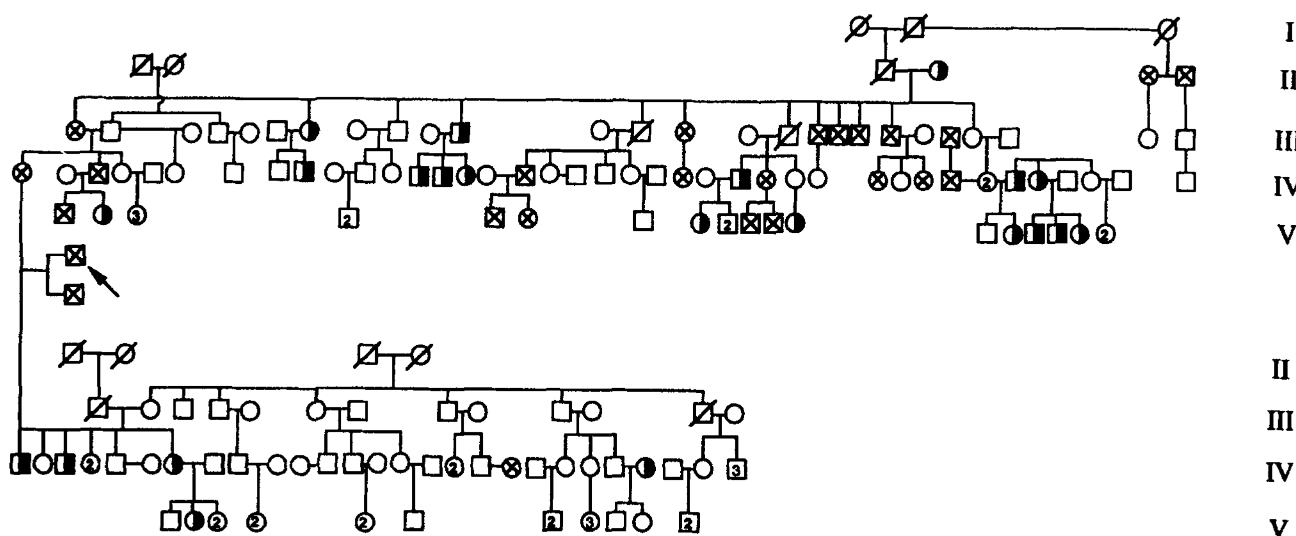

Fig. 1. Pedigree of a large French Canadian family with LPL deficiency. Heterozygotes for the P207L mutation are shown with semifilled circles or squares. The open circles or squares represent family relatives below the age of 40 who were included in this study. Arrow indicates the proband; (⧄, ⊘) deceased; (⊠, ⊗) not included (FH or data not available.

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TABLE 1. Characteristics of study cohort

	< 20 Years							20–40 Years							< 40 Years						
	Controls			Carriers				Controls			Carriers				Controls			Carriers			
	n	%	Mean±SD	n	%	Mean±SD	P	n	%	Mean±SD	n	%	Mean±SD	P	n	%	Mean±SD	n	%	Mean±SD	P
Females	19	0.56		5	0.42			17	0.44		36	0.5			36	0.5		9	0.43		
Males	15	0.44		7	0.58			20	0.54		5	0.56			35	0.49		12	0.57		
Total	34			12				37			9				71			21			
Age (years)	34		7.97±1.1	12		7.8±1.2	ns	37		30.4±3.7	9		34.4±2.4	ns	71		19.7±12.0	21		19.2±14.0	ns
BMI (kg/m2)	24		18.3±3.6	8		18.3±4.0	ns	36		23.5±4.08	9		24.1±2.33	ns	60		21.0±5	17		24±4.43	ns

BMI, body mass index; n, number in each group; ns, not significant, $P > 0.05$.

the transfer of apoC-III from VLDL to HDL and the notion that the ratio of apoC-III in HDL to that in VLDL functions as an index for the efficiency of lipolysis. Finally, the markedly reduced HDL_2 cholesterol values in carriers and the decrease in their LPA-I particle levels (containing apoA-I but not apoA-II) in the presence of normal LDL cholesterol levels is consistent with an enhanced susceptibility of carriers to atherosclerosis, although this remains to be determined.

METHODS

The family examined comprised 149 persons (**Fig. 1**) and was initially ascertained through a proband who was homozygous for the P207L mutation that occurs in exon 5 of the LPL gene. A total of 92 of these persons were below the age of 40 and were utilized in this study. Of these, 21 were shown to be heterozygous for the P207L mutation, with 71 being non-carriers. None of the subjects was on drug therapy modulating lipid levels (hypolipidemic drugs and/or hormonal therapy) and none of the participants had diabetes, thyroid, liver, or renal dysfunction. All subjects completed a targetted questionnaire and had height, weight, and blood pressure measured and gave informed consent following institutional guidelines. Another branch of this same family was found to carry a mutation in the gene for the LDL receptor in addition to the P207L. These individuals were excluded from the analysis presented here and have been the subject of a separate manuscript reported elsewhere (20).

Laboratory analysis

Venous blood was collected after an overnight fast in Vacutainer tubes containing EDTA. Immediately after collection, cells were separated from the plasma by centrifugation in a desktop centrifuge at 3,000 rpm for 20 min at room temperature. Multiple aliquots of plasma were frozen at -70°C for apolipoprotein assays. Although laboratory investigations could not be carried out on all the samples due to problems of sample insufficiency, no experimental or collection bias could be attributed to account for the differences recorded between the cohorts.

Lipoprotein analysis

Plasma lipoprotein fractions VLDL ($d < 1.006$ g/L); IDL ($1.006 < d < 1.019$ g/L); LDL ($1.019 < d < 1.063$ g/L) and HDL ($1.063 < d < 1.21$ g/L) were isolated by sequential ultracentrifugation in a Beckman 50.4 rotor using Quick-Seal tubes (21). Recovery of lipids in the lipoprotein fractions averaged 96% (range 92–102%) of the measured values in plasma. Lipid measurements in plasma and lipoprotein fractions were performed on an

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automated Technicon RA-1000 analyzer using enzymatic methods. Reagents for cholesterol and triglyceride measurements were purchased from Miles Diagnostics, for unesterified cholesterol from Boehringer-Mannheim, and for phospholipids from Wako Pure Chemicals. ApoB concentrations were measured in plasma, whereas LDL-apoB and apoA-I in the density fraction d > 1.006 g/L were measured by Laurell's rocket immunoelectrophoresis (22, 23). The assays for apoA-I and apoB were standardized with reference sera obtained from the Center for Disease Control, Atlanta, GA. The cholesterol concentration in the HDL subfractions, HDL_2 and HDL_3, were measured using density fraction, d > 1.006 g/L and after precipitation with low molecular weight dextran sulfate (Sochibo, France) as previously described (24). The cumulative coefficient of variation for the various lipid measurements is < 2% and for apoA-I and B, < 3%.

The immunoassays of apolipoproteins were performed by procedures previously described for apolipoprotein C-III (22, 25). ApoC-III was measured in whole serum (apoC-III-WS) and in heparin precipitated (apoC-III-HP) and heparin supernate (apoC-III-HS) lipoproteins, enabling the ratio of apoC-III-HS to apoC-III-HP (apoC-III ratio) to be calculated. ApoC-III-HS approximated the quantity of apoC-III in HDL whereas apoC-III-HP approximates that in VLDL + IDL. All the measurements were carried out in duplicate and repeated in duplicate when they disagreed by more than 5%. Repeat measurements were accepted when agreement was within 5%.

ApoE was measured by a noncompetitive enzyme-linked immunoabsorbent assay (ELISA) as described previously (26) using a monoclonal antibody that binds to apoE and to lipoprotein of human plasma containing apoE.

The apoA-I was measured by immunonephelometry on a Behring BNA nephelometer using the Behring antisera and standards. LpA-I particles were measured by rocket immunoelectrophoresis using Sebia kits and reagents (27). LpA-I–A-II levels were calculated by subtracting LpA-I from total apoA-I levels.

Statistics

The study groups were compared using analysis of variance (Anova). The effect of carrier status for mutation and age and their possible interaction were assessed by analysis of variance using the general linear models procedure. Where applicable, variables were logarithmically transformed to remove extreme skewness or nonparametric analysis of variance was used. Analyses were performed using the SAS statistical package version 6.04. All statistical procedures were carried out according to the standard text (28). The significance of *P* values was assessed taking into account the Bonferroni correction factor for multiple comparisons. For Tables 2–7, significance should be ascribed for *P* values < 0.03 and for Table 8, *P* < 0.02.

RESULTS

Demographics of the study cohort

The total number of controls and carriers and relative proportions between males and females were similar in controls and carriers of all ages. There were no differences in the body mass index (kg/m^2) in each of the groups. Controls and carriers were therefore appropriately matched for age and sex as well as total numbers (**Table 1**). Results were assessed for persons of different ages including 0–20, 20–40, and for all subjects below the age of 40 years.

Total cohort (0–40 years of age)

Plasma levels of lipids and lipoproteins. The mean total triglyceride level in carriers showed an increase (+69%; *P* = 0.056) when compared to the mean in nonheterozygous relatives. This reflected an increase in VLDL (+60%; *P* = 0.012), IDL (+40%; *P* = 0.001) and LDL triglyceride (+42%; *P* = 0.0001) respectively (**Table 2**).

TABLE 2. Plasma lipoprotein triglyceride levels

	< 20 Years							20 - 40 Years							<40 years						
	Controls			Carriers				Controls			Carriers				Controls			Carriers			
	n	Mean	SD	n	Mean	SD	P	n	Mean	SD	n	Mean	SD	P	n	Mean	SD	n	Mean	SD	P
		nmol/l			nmol/l				nmol/l			nmol/l				nmol/l			nmol/l		
Total TG	34	1.15	0.42	12	1.82	0.83	0.02	38	1.62	1.34	9	3.11	3.08	ns	71	1.4	1.03	21	2.37	2.14	0.06
VLDL TG	24	0.76	0.45	10	1.39	0.88	0.06	37	1.11	1.17	9	1.73	0.86	ns	61	0.97	0.97	19	1.55	0.86	0.01
IDL TG	24	0.05	0.02	10	0.06	0.03	ns	37	0.05	0.02	8	0.07	0.02	0.02	61	0.05	0.02	18	0.07	0.02	0.001
LDL TG	22	0.22	0.05	10	0.3	0.07	0.001	34	0.25	0.08	9	0.39	0.15	0.03	56	0.24	0.07	19	0.34	0.12	0.0001
HDL TG	24	0.15	0.06	10	0.16	0.05	ns	37	0.15	0.06	9	0.17	0.1	ns	61	0.15	0.06	19	0.17	0.08	ns

SD, standard deviation; ns, not significant, *P* > 0.05.

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TABLE 3. Plasma lipoprotein cholesterol levels

	< 20 Years							20 – 40 Years							<40 years						
	Controls			Carriers				Controls			Carriers				Controls			Carriers			
	n	Mean	SD	n	Mean	SD	P	n	Mean	SD	n	Mean	SD	P	n	Mean	SD	n	Mean	SD	P
		nmol/l			*nmol/l*				*nmol/l*			*nmol/l*				*nmol/l*			*nmol/l*		
Total chol	34	4.24	0.70	12	4.39	0.85	ns	37	4.94	0.85	9	5.48	1.21	ns	71	4.61	0.85	21	4.86	1.14	ns
VLDLchol	34	0.46	0.22	12	0.74	0.39	0.04	37	0.63	0.76	9	0.95	0.41	ns	71	0.55	0.57	21	0.83	0.41	0.03
IDL chol	24	0.07	0.02	10	0.13	0.10	0.02	37	0.10	0.07	8	0.18	0.16	0.04	61	0.09	0.06	18	0.15	0.13	0.007
LDL chol	34	2.60	0.67	12	2.63	0.55	ns	37	3.09	0.69	9	3.34	0.73	ns	71	2.86	0.72	21	2.93	0.71	ns
HDL chol	34	1.16	0.29	12	0.95	0.17	0.02	37	1.21	0.28	9	0.86	0.20	0.001	71	1.19	0.28	21	0.91	0.18	0.0001
HDL_2 chol	23	0.46	0.22	10	0.36	0.15	ns	37	0.43	0.22	7	0.22	0.10	0.02	60	0.44	0.21	17	0.30	0.15	0.01
HDL_3 chol	23	0.78	0.13	10	0.61	0.05	0.0004	37	0.78	0.16	7	0.73	0.09	ns	60	0.78	0.15	17	0.66	0.09	0.002
TC/HDLc	34	3.8	0.88	12	4.75	1.12	0.005	37	4.45	1.65	9	6.9	3.68	ns	71	4.15	1.37	21	5.7	2.73	0.02

SD, standard deviation; ns, not significant, $P > 0.05$.

Total cholesterol levels were similar in heterozygotes and controls (**Table 3**). However, statistically significant differences were seen in individual lipoprotein fractions. Heterozygotes had increases in VLDL (+51%; $P = 0.03$) and IDL (+67%; $P = 0.007$) cholesterol levels. While LDL cholesterol remained unchanged, HDL cholesterol was decreased in heterozygotes (-24%; $P = 0.0001$) (Table 3). This decrease was manifest in both the HDL_2 (-32%; $P = 0.01$) and HDL_3 (-15%; $P = 0.002$) fractions. Significant decreases were also recorded for HDL cholesteryl ester (CE), unesterified cholesterol (UC), and phospholipid (PL) (**Table 4A**). Calculation of the TC/HDLc ratio revealed a significant elevation in heterozygotes (+37%; $P = 0.02$) (Table 3). A total of 62% of carriers had triglycerides greater than the 90th percentile compared to 36% of controls ($P = 0.04$). Furthermore, a total of 62% of carriers compared to 22% of controls had HDL cholesterol values below the 10th percentile $P < 0.001$).

Composition of the lipoprotein particles. Heterozygotes had significant increase in VLDL and IDL lipids (CE, UC, TG, PL) when compared to controls (data not shown). The increase in VLDL-CE however did not reach significance levels ($P = 0.08$). Despite this increase in lipids in these lipoprotein fractions, the relative proportion of each lipid constituent, expressed as the percentage of total lipid in the lipoprotein, was maintained in most cases (**Table 5**). The percentage of VLDL-UC, however, was decreased, although not significantly ($P = 0.056$), while the percentage of IDL-PL was significantly decreased ($P = 0.05$). In contrast to the relative homogeneity of the VLDL and IDL lipoproteins in the carriers and controls, the lipid composition of LDL and HDL

TABLE 4. Lipid composition of HDL

	< 20 Years							20 – 40 Years							<40 years						
A	Controls			Carriers				Controls			Carriers				Controls			Carriers			
	n	Mean	SD	n	Mean	SD	P	n	Mean	SD	n	Mean	SD	P	n	Mean	SD	n	Mean	SD	P
		nmol/l			*nmol/l*				*nmol/l*			*nmol/l*				*nmol/l*			*nmol/l*		
CE	24	1.03	0.23	10	0.83	0.14	0.014	37	1.01	0.21	8	0.78	0.12	0.008	61	1.02	0.22	18	0.81	0.13	0.0002
UC	24	0.23	0.366	10	0.15	0.45	0.0001	37	0.21	0.07	8	0.14	0.03	0.001	61	0.22	0.07	18	0.14	0.03	0.0001
TG	24	0.15	0.06	10	0.16	2.68	ns	37	0.15	0.06	8	0.17	0.10	ns	61	0.15	0.06	18	0.17	0.08	ns
PL	24	1.08	0.25	10	0.86	0.88	0.003	37	1.03	0.24	8	0.74	0.12	0.0001	61	1.05	0.25	18	0.61	0.14	0.0001
B		*% total lipid*			*% total lipid*				*% total lipid*			*% total lipid*				*% total lipid*			*% total lipid*		
CE	24	39.26	2.89	10	38.71	1.736	ns	37	40.10	2.48	8	40.86	1.29	ns	61	39.77	2.66	18	39.67	1.86	ns
UC	24	5.47	0.83	10	4.19	0.45	0.001	37	4.96	0.88	8	4.52	0.69	ns	61	5.16	0.89	18	4.34	0.58	0.001
TG	24	8.03	2.48	10	10.51	2.68	0.01	37	8.14	2.60	8	9.97	1.35	ns	61	8.09	2.53	18	10.27	2.15	0.001
PL	24	47.24	1.64	10	46.59	0.88	ns	37	46.81	1.43	8	44.65	1.23	0.0003	61	46.98	1.52	18	45.72	1.42	0.003

SD, standard deviation; CE, cholesteryl ester; UC, unesterified cholesterol; TG, triglyceride; PL, phospholipid; ns, not significant.

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TABLE 5. Lipid composition of VLDL and LDL particles

A	< 20 Years							20 - 40 Years							<40 years						
	Controls			Carriers				Controls			Carriers				Controls			Carriers			
	n	Mean	SD	n	Mean	SD	P	n	Mean	SD	n	Mean	SD	P	n	Mean	SD	n	Mean	SD	P
		nmol/l			*nmol/l*				*nmol/l*			*nmol/l*				*nmol/l*			*nmol/l*		
VLDL																					
CE	24	14.48	2.26	10	14.80	2.15	ns	37	14.34	3.58	8	14.82	2.29	ns	61	14.39	3.11	18	14.81	2.15	ns
UC	24	6.12	0.89	10	5.57	0.58	ns	37	6.11	1.04	8	5.97	0.55	ns	61	6.11	0.98	18	5.75	0.59	0.056
TG	24	60.02	2.25	10	60.930	3.17	ns	37	60.70	4.86	8	60.08	3.68	ns	61	60.43	4.03	18	60.55	3.33	ns
PL	24	19.39	1.63	10	18.70	1.01	ns	37	18.85	2.99	8	19.13	1.27	ns	61	19.06	2.54	18	18.89	1.12	ns
LDL																					
CE	22	52.58	2.59	10	52.20	1.66	ns	35	52.30	2.61	8	52.29	3.38	ns	57	52.14	2.59	18	52.24	2.48	ns
UC	22	9.78	0.73	10	8.92	0.88	0.007	35	10.10	1.16	8	9.19	1.46	ns	57	9.98	1.02	18	9.04	1.14	0.001
TG	22	8.45	2.82	10	10.91	1.97	0.02	35	8.06	2.38	8	10.35	2.28	0.018	57	8.21	2.54	18	10.66	2.07	0.0004
PL	22	29.18	1.16	10	27.97	1.12	0.01	35	29.54	1.85	8	28.18	2.87	ns	57	29.40	1.62	18	28.06	2.01	0.005

SD, standard deviation; CE, cholesteryl ester; UC, unesterified cholesterol; TG, triglyceride; PL, phospholipid; ns, not significant.

was clearly altered in heterozygotes (Tables 4B and 5). In both cases, the percentage of PL and UC in heterozygotes was significantly reduced (PL: $P < 0.001$; UC: $P < 0.005$) while the percentage of TG was markedly increased ($P < 0.001$). The percentage of CE, both in LDL and HDL, was essentially maintained between carriers and controls.

Plasma apolipoprotein levels. Carriers had a significant decrease (-13%; $P = 0.0009$) in plasma total apoA-I and an increase (+11%; $P = 0.04$) in total apoB with a moderate increase in LDL apoB (**Table 6**). There were no statistically significant differences in total apoE, or in HDL and non-HDL apoE levels. The total apoC-III levels were moderately increased in carriers although they failed to reach statistical significance (12.82 vs. 10.94 mg/dl). Carrier apoC-III levels were significantly increased in the heparin-precipitable lipoprotein fractions, i.e., in VLDL and IDL (apoC-III-HP) ($P = 0.001$). As a consequence, the ratio of total apoC-III to VLDL apoC-III was decreased in carriers when compared to controls ($P = 0.0001$).

Lipoprotein particles defined by their apolipoprotein composition (LpA-I) were significantly decreased (-21%; $P = 0.00001$) in the carriers as was the concentration of LpA-I~A-II particles (-10%; $P = 0.02$) but to a lesser extent (Table 7).

Specific age cohorts (< 20 yr and 20–40 yr)

The study cohort was subdivided into age groups of individuals above (20–40) and below 20 years of age (< 20) to study the modulating effect of age on the phenotypic expression of the P207L mutation. In general, significant differences between controls and carriers in the < 20 cohort were also evident in the 20–40 age group. However, some age-modulating effects on the phenotypic expression of heterozygosity for LPL deficiency were apparent, particularly for HDL_2 and HDL_3. Carriers aged 20–40 showed a significant reduction in HDL_2 levels ($P = 0.02$) which was not seen in the < 20 cohort. Conversely, the significant differences in HDL_3 values in the < 20 cohort ($P = 0.0004$) were not evident in the 20–40 cohort. These results clearly demonstrate that age can modulate the phenotypic expression of heterozygosity for LPL deficiency particularly for HDL values.

DISCUSSION

This family with the P207L mutation in the LPL gene has provided a unique opportunity to determine the phenotypic expression of heterozygotes for LPL deficiency before the age of 40 years in a single kindred. The family had its origins in a small town in Eastern Quebec and all members followed a similar diet. In addition, there were sufficient males and females below the age of 40 to directly address the question as to the phenotypic expression of LPL deficiency before the age of 40 years. This study conclusively shows that significant alterations in plasma lipoproteins and apolipoproteins occur before this age in heterozygotes for the P207L mutation. This family has also provided a unique opportunity to address in vivo the effects of partial defects in lipolysis.

This study supports reports of other kindreds with molecularly defined LPL deficiency (14, 15). However, in contrast to one study of the G188E mutation, which

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TABLE 6. Plasma levels of apolipoproteins and distribution in different density fractions

A	< 20 Years							20 - 40 Years							<40 years						
	Controls			Carriers				Controls			Carriers				Controls			Carriers			
	n	Mean	SD	n	Mean	SD	*P*	n	Mean	SD	n	Mean	SD	*P*	n	Mean	SD	n	Mean	SD	*P*
		mg/dl			*mg/dl*				*mg/dl*			*mg/dl*				*mg/dl*			*mg/dl*		
Total apoA-I	23	1.45	0.26	10	1.30	0.10	ns	34	1.48	0.18	8	1.26	0.19	0.002	57	1.47	0.21	18	1.28	0.14	0.0009
Total apoB	34	0.91	0.18	12	0.97	0.25	ns	37	0.96	0.18	9	1.13	0.21	0.02	71	0.94	0.18	21	1.04	0.24	0.04
LDL apoB	242	0.78	0.17	10	0.81	0.19	ns	37	0.85	0.16	9	1.01	0.19	0.02	61	0.83	0.17	19	0.90	0.21	0.05
Total apoE	23	0.09	0.03	10	0.09	0.03	ns	34	0.09	0.05	7	0.11	0.03	ns	57	0.09	0.04	17	0.10	0.03	ns
LpE-HDL	23	0.05	0.21	10	0.01	0.01	ns	34	0.01	0.01	8	0.02	0.01	ns	57	0.03	0.13	18	0.02	0.01	ns
LpE-non-HDL	23	0.08	0.03	10	0.08	0.03	ns	34	0.07	0.05	7	0.08	0.04	ns	57	0.07	0.04	17	0.08	0.03	ns
Total apoC-III	15	10.79	2.06	5	12.32	3.21	ns	33	11.02	4.34	8	13.13	4.66	ns	48	10.94	3.76	13	12.82	4.03	ns
ApoC-III-HS	15	6.85	1.67	5	5.88	0.75	ns	33	6.89	1.96	8	5.94	1.40	ns	48	6.88	1.86	13	5.92	1.15	ns
ApoC-III-HP	15	2.26	0.60	5	5.14	3.24	ns	33	2.99	2.94	8	5.46	2.81	ns	148	2.76	2.47	13	5.34	2.85	0.001
ApoC-III- ratio	15	2.23	1.08	5	1.70	1.15	0.004	33	4.14	3.48	88	1.32	0.60	0.004	48	3.86	2.96	13	1.46	0.83	0.0001

primarily manifests with changes in lipoprotein values after age 40, our report clearly shows evidence for changes in lipoprotein values at an earlier age. This could reflect other genetic factors operating in these two families, varying environments, or differential effects of various mutations in the LPL gene.

As a whole, heterozygotes were found to manifest a mild hypertriglyceridemia involving elevated VLDL and IDL levels, triglyceride-enriched LDL and HDL, and decreased HDL cholesterol. The VLDL and IDL fractions were also found to be enriched in apoC-III. These differences clearly indicate a crucial role for LPL activity in the metabolism of lipoprotein triglyceride and the mediation of lipid and apolipoprotein transfer amongst different plasma lipoproteins.

It has previously been shown in vitro that lipolysis enhances (29) the activity of CETP, resulting in augmented transfer and exchange of HDL CE. This has been shown to be due to the enhanced ability of VLDL remnants to accept HDL CE related to the accumulation of fatty acid in the VLDL surface during lipolysis (30). If this process necessitated fully functional LPL activity, one would expect that heterozygotes for LPL deficiency would have a significant increase in the percentage of CE in the HDL fraction and a decrease in the percentage of CE in the VLDL fraction. However, our results indicate that even with an increased number of VLDL and IDL particles, heterozygotes have normal proportions of CE in VLDL, IDL, and HDL. This suggests that approximately half the normal LPL activity is still sufficient to result in normal transfer of CE between HDL and VLDL.

The HDL fraction of heterozygotes showed a significant decrease in UC and PL and in CE. This provides in vivo evidence that lipolytic activity of LPL is crucial for the removal of the surface constituents, PL and UC, from VLDL. In the presence of partial defects in LPL activity, the phospholipids and unesterified cholesterol remain within the VLDL particle and are not transferred as efficiently to HDL. As a result there is a marked decrease in HDL, UC and PL. These in vivo observations complement the in vitro data that has shown that during lipolysis of LPL, surface constituents leave VLDL simultaneous with hydrolysis of core triglycerides (31). The concomitant decrease in the HDL CE of heterozygotes is indicative of near normal CETP activity in the presence of only a partial impairment of lipolysis.

Heterozygotes were also found to have triglyceride-enriched LDL. It has previously been shown in vitro that triglyceride-enriched LDL is a cholesterol-poor lipoprotein and down-regulates LDL receptors in cultured fibroblasts less efficiently than normal LDL (32). If this were happening in vivo, one would expect that TG-enriched LDL particles would be cleared from plasma at an accelerated rate and that LDL cholesterol levels might decrease. However, LDLc levels are not significantly altered in these heterozygotes. In the presence of a partial decrease of LPL activity, the down-regulation of the LDL receptor activity by a triglyceride-rich LDL may be counterbalanced by the decreased effect of LPL in mediating the uptake of LDL. As such, in these patients, LDL cholesterol does not show any significant changes from a control level.

The protein composition of lipoproteins is also altered in these heterozygotes for LPL deficiency. In particular, apoB levels are increased (Table 6) and LDL becomes relatively enriched with apoB. It has previously been shown that in the presence of hypertriglyceridemia, LDL is relatively enriched with proteins and triglycerides (33). The finding of increased LDL-

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TABLE 7. Plasma levels of apoA-conataining particles

A	< 20 Years							20 – 40 Years							<40 years						
	Controls			Carriers				Controls			Carriers				Controls			Carriers			
	n	Mean	SD	n	Mean	SD	P	n	Mean	SD	n	Mean	SD	P	n	Mean	SD	n	Mean	SD	P
		mg/dl			*mg/dl*				*nmol/l*			*nmol/l*				*nmol/l*			*nmol/l*		
LpA-I	23	0.43	0.10	10	0.34	0.04	0.01	34	0.43	0.07	8	0.33	0.06	0.001	57	0.43	0.08	18	0.34	0.04	0.00001
LpA-I:A-II	23	1.02	0.19	10	0.96	0.09	ns	34	1.06	0.15	8	0.92	0.17	0.03	57	1.04	0.17	18	0.94	0.13	0.02

SD, standard deviation; ns, not significant, $P = 0.05$.

apoB in heterozygotes for LPL deficiency is consistent with prior hypotheses that decreased LPL activity may be associated with a lipoprotein phenotype consistent with familial combined hyperlipidemia (34). Recently, different mutations in the LPL gene have been found in patients with FCHL. These include the $Asn^{291} \rightarrow {}^{Ser}$ (N291S) mutation seen in 11% of patients with FCHL (12) and mutations in the promoter region of the LPL gene (35).

There have been no reports of apoC-III levels or their distribution in heterozygotes for LPL deficiency. It has been proposed that the apoC-III ratio (apoC-III-HS/apoC-III-HP) can be used as a marker to estimate the distribution of apoC peptides between HDL and VLDL (36–38) which is determined by the metabolism of triglyceride-rich lipoproteins. A decrease in the C-III ratio indicates a shift in the distribution of apoC-III from HDL to VLDL + LDL and would be indicative of some defect or delay in VLDL clearance. Heterozygotes for LPL deficiency had a marked increased in C-III in VLDL (apoC-III-HP) which was evident before the age of 20 and also seen in the cohort of 20–40 years. Consequently, the apoC-III ratio was markedly decreased in carriers at an early age.

A primary risk factor predicting the probability of progression of coronary artery lesions is the content of apoC-III in HDL, with a decrease in the C-III ratio being associated with progression of atherosclerotic lesions (39, 40). Furthermore, apoC-III in VLDL is associated with denser, smaller VLDL subclasses that are particu-

TABLE 8. HDL cholesterol in males and females

A	< 20 Years							20 – 40 Years							<40 years						
	Controls			Carriers				Controls			Carriers				Controls			Carriers			
	n	Mean	SD	n	Mean	SD	P	n	Mean	SD	n	Mean	SD	P	n	Mean	SD	n	Mean	SD	P
		nmol/l			*nmol/l*				*nmol/l*			*nmol/l*				*nmol/l*			*nmol/l*		
HDL chol																					
All	34	1.16	0.29	12	0.95	0.17	0.02	37	1.21	0.28	9	0.86	0.20	0.001	71	1.19	0.28	21	0.91	0.18	0.0001
Males	15	1.17	0.26	7	0.96	0.17	ns	20	1.1	0.23	5	0.76	0.2	0.01	35	1.13	0.25	12	0.89	0.2	0.004
Females	19	1.15	0.32	5	0.92	0.17	ns	17	1.35	0.27	4	0.97	0.17	0.017	36	1..25	0.31	9	0.94	0.16	0.007
P#		ns			ns				0.005			ns				ns			ns		
HDl$_2$ chol																					
All	23	0.46	0.22	10	0.36	0.15	ns	37	0.43	0.22	7	0.22	0.10	0.015	60	0.44	0.21	17	0.30	0.15	0.01
Males	9	0.42	0.19	6	0.34	0.17	ns	20	0.32	0.13	3	0.16	0.05	ns	29	0.35	0.16	9	0.28	0.16	ns
Females	14	0.49	0.23	4	0.4	0.13	ns	17	0.57	0.22	4	0.26	0.12	0.014	31	0.53	0.22	8	0.33	0.14	0.02
P#		ns			ns				0.0001			ns				0.0006			ns		
HDl^3 chol																					
All	23	0.78	0.13	10	0.61	0.05	0.0004	37	0.78	0.16	7	0.73	0.09	ns	60	0.78	0.15	17	0.66	0.09	0.002
Males	9	0.81	0.12	6	0.63	0.03	0.004	20	0.78	0.15	3	0.76	0.09	ns	29	0.79	0.14	9	0.68	0.08	0.03
Females	14	0.76	0.13	4	0.59	0.07	0.023	17	0.78	0.17	4	0.71	0.09	ns	31	0.77	0.15	8	0.65	0.1	0.03
P#		ns			ns				ns			ns				ns			ns		

SD, standard deviation; ns, not significant, $P = 0.05$

[a] Bonferroni adjusted P value = 0.02 (significance).

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larly atherogenic (39, 41, 42). In the presence of partial defects of lipolysis, VLDL particles enriched in apoC-III circulate for a longer period of time and there is increased probability of contact between these lipoprotein particles and their arterial walls. A decreased apoC-III ratio in heterozygotes for LPL deficiency is certainly consistent with a lipoprotein profile associated with an increased susceptibility to atherosclerosis. This is further strengthened by additional evidence for an enhanced susceptibility for atherosclerosis based on decreased levels of apoA-I (42–44) in carriers for LPL deficiency, which importantly is again evident before the age of 20. The TC/HDLc ratio was also significantly elevated in all carriers (Table 3) but particularly in the < 20 yr age group. This ratio has been shown by multivariate analysis to be an important indicator of the likelihood of regression of atheroma (40).

In this study HDL particles containing apoA-I (LpA-I) were significantly decreased in carriers before the age of 20. HDL particles containing both LpA-I and A-II were also reduced but to a lesser degree. This is consistent with LpA-I levels being in part dependent on catalytic activity. A prior study has also reported a significant correlation between LpA-I and lipoprotein lipase activity (45). In vitro studies of the binding of LpA-I and LpA-I–A-II to various cells has suggested that LpA-I particles promote cholesterol efflux from cells (46). Recent studies have also indicated that the presence of coronary artery disease is inversely correlated with levels of LpA-I but not LpA-I–A-II (47, 48). A more recent study of lipid and lipoprotein parameters in adolescent children of parents with premature CAD found that plasma LpA-I level was the single best discriminating factor between cases and controls (48). A decrease in the C-III ratio together with a marked decrease in apoA-I and decrease in LpA-I provides biochemical evidence for an increased susceptibility of these heterozygotes to increasing risk for atherosclerosis. Clearly, additional in vivo studies both in humans and animals are needed to assess whether heterozygotes for LPL deficiency are, as would be predicted, at increased risk for atherosclerotic events. Recently described animal models (mouse and cat) for LPL deficiency will allow more direct assessment of this hypothesis (49, 50).

In a prior study of LPL deficiency in heterozygotes for the G188E mutation, it was proposed that lipoprotein abnormalities discriminating between carriers and non-carriers were only observed after the age of 40 yr (14). This study of the P207L carriers, however, shows that significant disturbances of lipoproteins and apolipoprotein composition of lipoproteins are present in many instances before the age of 20 yr. However, there is still evidence that other factors, such as gender, influence the phenotypic expression of this particular mutation. For example, HDL cholesterol levels are significantly decreased before the age of 20 yr in the group of carriers as a whole compared to controls. However, for males these changes are most marked in the cohort between 20–40 years (**Table 8**). While HDL levels in female carriers increase from a mean of 0.92 mmol/l before the age of 20 to a level of 0.97 between the ages of 20–40 yr (a trend similar to that seen in controls), the HDL levels in male carriers for this mutation show a significant decrease after the age of 20 yr from a level of 0.96 to 0.78 mmol/l. This is primarily due to a marked reduction (> 50%) in HDL_2 levels in male heterozygotes. In contrast, the HDL_3 levels continue to increase after the age of 20 yr in male heterozygotes. Thus, there appears to be a sex-modulated effect on HDL_2 cholesterol levels in heterozygotes for this mutation with males over the age of 20 yr having a particularly decreased HDL_2 cholesterol level.

In summary, this study provides in vivo evidence for significant disturbances in lipoprotein metabolism in heterozygotes for LPL deficiency and clearly supports the crucial role of LPL and lipolysis in determining the lipid composition of lipoproteins. In addition, these disturbances in lipoprotein metabolism are evident in most instances before the age of 20 and would predict an increased susceptibility of the P207L heterozygotes to atherosclerosis.

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