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Research Article

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Heterozygous Lipoprotein Lipase Deficiency Due to a Missense Mutation as the Cause of Impaired Triglyceride Tolerance with Multiple Lipoprotein Abnormalities

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Abstract

In 16 members of two Austrian families affected by a missense mutation at codon 188 of the lipoprotein lipase (LPL) gene (8 heterozygous and 8 normal subjects), carrier status for the mutation as determined by DNA analysis was related to LPL activity in postheparin plasma, to the magnitude of postprandial lipemia, and to concentration, composition, and size of the major lipoprotein classes of postabsorptive plasma. Carriers exhibited clearly reduced LPL activity, normal fasting triglycerides, but pronounced postprandial lipemia. The carriers' impaired triglyceride tolerance, as evident in the postprandial state of challenge only, was associated with a fasting lipoprotein constellation characterized by (*a*) enrichment of HDL_2 with triglycerides, (*b*) reduced HDL_2-cholesterol, (*c*) enrichment of VLDL and intermediate density lipoprotein (IDL) with cholesteryl esters, (*d*) elevated IDL levels, and (*e*) small-sized LDL. Within any given individual, the degrees of expression of these characteristics were quantitatively and continuously related with each other as well as with the magnitude of lipemia and with LPL activity. (*J. Clin. Invest.* 1993. 91:448–455.) Key words: high-density lipoprotein 2 • intermediate-density lipoprotein • low-density lipoprotein • lipoprotein lipase • postprandial lipemia

Introduction

Lipoprotein lipase (LPL[1] EC 3.1.1.34) hydrolyzes lipoprotein triacylglycerols to monoacylglycerols and fatty acids and in this way unloads 70–150 g of triglycerides per day from chylomicrons and VLDL (1–4). The human enzyme is encoded by a gene spanning ~ 30 kb of chromosome 8, with its coding sequence split into 10 exons, and is produced mainly in fat and muscle cells (4–7). To allow interactions between the enzyme and circulating triglyceride (TG)-rich lipoproteins, LPL is secreted from its sites of synthesis and attaches to heparan sulfate anchors of the vascular endothelium (3), the site of its action.

Numerous mutations at the LPL gene locus have been defined (for review see references 8 and 9), disrupting the function of the enzyme at the levels of synthesis, endothelial attachment, or catalytic competence (10). The nature of the mutations described includes partial gene duplication, major deletion, frameshift insertion, and several point mutations, most commonly missense substitutions with a tendency to cluster in exon 5 (8, 9). An example of the latter is the single-base transition from guanine to adenine at cDNA position 818 in codon 188 (11–13), which substitutes glutamic acid for glycine and results in the synthesis and secretion of a catalytically inactive enzyme. Inheritance of two defective alleles of the LPL gene causes the inability to process TG-rich lipoproteins and leads to a clinical syndrome characterized by vast TG elevation, fasting chylomicronemia, lipid deposition in dermal, hepatic, and splenic macrophages, and bouts of pancreatitis (14). Carriage of only one defective LPL gene, on the contrary, is not usually heralded by gross phenotypic stigmata such as chylomicronemia, xanthomata, or episodes of abdominal pain. However, heterozygous carriers have only half-normal LPL activities (15) which may not suffice to keep the plasma TG concentration within normal limits when stress is placed on the plasma lipid transport system. For instance, heterozygotes are prone to moderate fasting hypertriglyceridemia if secondary factors such as obesity, hyperinsulinemia, or use of lipid-raising agents are superimposed on the genetic defect (16).

We report here that, even in the absence of such secondary factors, a trivial challenge like postprandial lipemia can uncover heterozygous carriage of a defective LPL gene. In members of two Austrian families affected by the missense mutation at codon 188 (13), the gene defect was clearly manifested in the postprandial state as the diminished ability to clear the absorbed TG load from plasma. We propose to refer to this metabolic handicap as "impaired TG tolerance" in order to (*a*) distinguish it from fasting hypertriglyceridemia, (*b*) to emphasize that a tolerance test (the oral fat load) is required for diagnosis, and (*c*) to allude to the analogy between this condition and that of "impaired glucose tolerance." Although the carriers' condition of impaired TG tolerance became overt in the postprandial state only, it left typical marks on all lipoprotein classes in fasting plasma; pronounced postprandial lipemia was associated with TG-enriched HDL, low HDL_2-cholesterol, cholesterol-enriched VLDL and intermediate density lipoprotein (IDL), high IDL levels, and small LDL particles. Because these stigmata are all components of a firm constellation, we propose to call this constellation "the syndrome of impaired TG tolerance." Impaired TG tolerance and its associated syndrome can probably be caused by many molecular defects; heterozygous LPL deficiency described in this report may serve as the first clearly defined example.

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1. *Abbreviations used in this paper:* CETP, cholesteryl ester transfer protein; IDL, intermediate-density lipoprotein; HL, hepatic lipase; LPL, lipoprotein lipase; RP, retinyl palmitate; S_f, flotation rate; TG, triglyceride.

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Methods

Study subjects. A total of 16 members of the two Austrian families affected by the missense mutation at codon 188 of the LPL gene (13) gave informed consent to be reexamined. Family members had been genotyped earlier. Briefly, genomic fragments containing exon 5 of the LPL gene were amplified by polymerase chain reaction and digested with the restriction endonuclease Ava II. The GGG-to-GAG base transition at codon 188 abolishes one of the two Ava II sites present in exon 5 of the wild-type allele, leading to different restriction fragment patterns which were used to identify carriers and noncarriers (12, 13).

All eight family members above the age of 10 yr who were heterozygous carriers of the mutation (four females and four males, ranging in age from 10 to 69 yr) were studied and compared with eight unaffected relatives (six females and two males, ranging in age from 11 to 63 yr). All subjects were on essentially the same Western diet. With the sole exception of three noncarriers who used contraceptive steroids, none of the study subjects took medication or used alcohol regularly. No subject had clinical or biochemical evidence of diabetes or cardiac, hepatic, renal, or endocrine disease. The plasma levels of glucose, insulin, aspartate aminotransferase, alanine aminotransferase, GGT, creatinine, blood urea nitrogen, uric acid, and thyroid stimulating hormone were within normal limits and did not differ between carriers and noncarriers (Table I). All individuals were normotensive and within ideal body weight range (Table I).

Plasma lipids, lipoproteins, and apolipoproteins. During a first visit, postabsorptive plasma was collected after an overnight fast. Cholesterol, TGs, and apolipoproteins (apo) A-I and B were quantified by automated colorimetric and turbidimetric procedures, respectively (Boehringer Mannheim Gmb H, Mannheim, FRG); apoA-II levels were estimated by single radial immunodiffusion (Immuno AG, Vienna, Austria). ApoE phenotypes were determined by isoelectric focusing of delipidated plasma, Western blotting, and immunostaining (17).

Two 10-ml aliquots of postabsorptive plasma were subjected to rate zonal ultracentrifugation in a Ti-14 rotor (Beckman Instruments, Inc., Klosterneuburg, Austria) under conditions to isolate HDL_2 and HDL_3 and the major lipoprotein classes with density < 1.063 g/ml, respectively (18, 19). Zonal rotor fractions were analyzed for protein (20), phospholipids (21), total and unesterified cholesterol, and TGs (Boehringer Mannheim). Plasma concentrations of lipoproteins and constituents thereof were calculated from these data. Stokes diameters of LDL and HDL subfractions were estimated after electrophoresis under nondenaturing conditions on PAA 2/16 and PAA 4/30 polyacrylamide gradient gels (Pharmacia LKB Biotechnology, Uppsala, Sweden), respectively (22, 23).

Postprandial lipemia. Immediately after sampling of postabsorptive plasma, the probands ingested a liquid fatty meal as detailed previously (24). The test meal consisted per square meter body surface of 65.0 g fat with a P/S ratio of 0.06, 24 g of carbohydrate, 4.75 g of protein, 240 mg of cholesterol, and 60,000 U of aqueous vitamin A (Oleovit-A, Laevosan, Linz, Austria) to biosynthetically label intestinal lipoproteins (25–28). TGs were determined at 2, 4, 6, 8, and 10 h postprandially; the magnitude of lipemia was quantified as the area under the postprandial TG curve normalized to the fasting level (24). Aliquots of the postabsorptive and all postprandial plasma specimens were overlayered with equal volumes of 0.9% saline and centrifuged in a SW-55 rotor (Beckman Instruments, Inc.) at 40,000 rpm for 30 min to float lipoproteins with $S_f > 1{,}000$ into the overlay, and in this way separate a "chylomicron" and a "remnant" fraction. Lipids contained in these fractions were extracted and chromatographed in an HPLC system equipped with a 5-μm particle ODS Ultrasphere column (both Beckman Instruments, Inc.). The column effluent was monitored at 313 nm, and retinyl palmitate (RP) was quantified by integrating the area under the RP peak (Beckman System Gold Version 5.1 software) in comparison to that of an external RP standard (Sigma Chemical Co., St. Louis, MO).

Lipases and cholesteryl ester transfer protein. During a second visit, probands in the postabsorptive state were injected intravenously with 2,280 U/m^2 heparin (Novo Industri A/S, Copenhagen, Denmark) to release LPL and hepatic lipase (HL) into the circulation, and postheparin plasma was collected after 10 min. Sonicated emulsions of [9,10-^{3}H] oleic acid-labeled trioleoylglycerol (Amersham Corp., Arlington Heights, IL) in phosphatidylcholine and gum arabic were employed as substrates for estimation of LPL and HL activity, respectively (13, 29). To LPL assay mixtures, heat-inactivated fasted rat serum was added as a source of apoC-II; HL was inhibited by goat anti–human HL IgG. For assay of HL, LPL was suppressed by raising the NaCl concentration of the incubation mixture to 1 M and omitting the source of apoC-II. Lipase activities were measured by incubating for 30 min at 25°C and at a pH of 8.5 (13, 29). Activity is expressed in milliunits (mU), which correspond to 1 nmol of fatty acids released per minute.

The activity of cholesteryl ester transfer protein (CETP) was quantified as the transfer of [1-^{14}C] oleate-labeled cholesteryl esters (Amersham Corp.) from exogenous LDL to exogenous HDL, mediated by a fraction of the respective probands' postabsorptive plasma which had been depleted of endogenous VLDL and LDL (30). Assay mixtures were incubated for 16 h at 37°C and at a pH of 7.4 in the presence of 2 mM 5,5′-dithiobis(nitrobenzoic acid) (Sigma Chemical Co.) to inhibit the de novo formation of cholesteryl esters. Radioactivity incorporated into HDL was quantified after precipitation of LDL. CETP activity is expressed in nanomoles cholesteryl ester transferred to HDL per hour per milliliter plasma.

Statistics. Although all eligible members of the two families participated in the study, a perfect match of our study subjects with respect to age and sex was not possible (Table I). In a comparable investigation, Wilson et al. (16) attempted to solve this problem by adjusting their raw data on cholesterol, TGs, and VLDL-, LDL-, and HDL-cholesterol to male age 40–44-yr values from the Lipid Research Clinics Population Studies Data Book (31). We, however, could not adopt this approach, because population data were not available for most variables of interest in this study, including, for instance, the magnitude of lipemia, levels of lipoproteins and components thereof, and lipase activities. Our approach, therefore, was to statistically control for age and sex where appropriate.

Statistical analyses were performed with the help of SPSS-X Release 4 software (SPSS Inc., Chicago, IL) (32, 33). To satisfy the assumptions of normality and homogeneity of variances in carriers and noncarriers, variables were transformed where necessary. Appropriate transformations were selected on the basis of graphical techniques, skewness and kurtosis statistics, and the Shapiro-Wilks and Lilliefors statistics to ascertain normality, and the Levene statistic to ascertain the homogeneity of variances (32).

Variables were compared between groups by analysis of variance. For each set of variables presented in one table (Tables II–V), multivariate analysis of variance (33) was performed first to reject the null

Table I. Clinical Data of Study Subjects

	Carriers (n = 8)	Noncarriers (n = 8)	P value
Age (*yr*)	49.5 (22.9)	27.3 (17.9)	0.048
Body mass index (*kg/m²*)	24.1 (3.7)	23.0 (4.6)	0.601
Glucose (*mg/dl*)	97.9 (6.3)	95.1 (10.2)	0.529
Insulin (*μU/ml*)	7.8 (2.0)	6.8 (2.1)	0.329
Uric acid (*mg/dl*)	4.6 (1.1)	4.1 (0.7)	0.288
Thyroid-stimulating hormone (*mU/liter*)	1.45 (0.97)	1.43 (0.81)	0.956

Results are means (SD) as calculated from untransformed data. The normal ranges are 70–110 mg/dl for glucose, 5–20 μU/ml for insulin, 2.5–7.0 mg/dl for uric acid, and 0.1–4.0 mU/l for thyroid-stimulating hormone. P values were obtained by analysis of variance, using untransformed data.

hypothesis of no difference between carriers and noncarriers. To define the individual variables accounting for the multivariate differences, univariate significance tests were performed using two different designs: a one-way design for comparison of carriers and noncarriers, and an extended one-way design including age as covariate to remove potentially confounding effects of age differences (32). Postprandial TG and RP curves were evaluated by repeated measures analysis of variance, employing orthogonal polynomial contrasts (33).

Relationships between variables are presented as second-order partial correlation coefficients controlling for age and sex (32); correlations were computed for the entire set of 16 study subjects. Scatterplotts identifying heterozygous and normal individuals were used to assure that the associations were linear and valid for both groups of study subjects; residuals were analyzed for normality and constant variance (32).

Results

Postprandial versus postabsorptive metabolism of TG-rich lipoproteins. Heterozygous carriers of the mutant LPL gene displayed clearly reduced postheparin plasma LPL activities, averaging 38% of those of noncarriers (Table II); individual measurements did not overlap between the two sets of study subjects (LPL activity ranges: 87–209 mU/ml in carriers vs. 216–507 mU/ml in noncarriers). Among the routinely measured fasting lipid and apolipoprotein concentrations (Table III), the distinctive reflection of this metabolic handicap was a lower HDL_2-cholesterol level rather than elevated TGs. Accordingly, zonal ultracentrifugation (Fig. 1) and nondenaturing gradient gel electrophoresis (data not shown) revealed a predominance of the small and dense HDL subclasses in carriers.

Low HDL_2-cholesterol levels are strongly indicative of pronounced postprandial lipemia or impaired TG tolerance (24, 34). We therefore proceeded to investigating whether carriage of the mutant LPL gene would become apparent more clearly in the postprandial state, when the pathway of plasma TG clearance was loaded. This was indeed the case (Fig. 2). The mean magnitude of postprandial lipemia in carriers exceeded more than twice that of noncarriers (903.3 vs. 423.5 mg/dl · 10 h, $P = 0.006$; $P = 0.025$ when age was included as covariate). Beyond that, the shapes of the average postprandial TG curves appeared to differ; the rising part of the carriers' curve displayed increased slope and prolonged duration, leading to higher and later TG peaks on one hand and to particularly wide concentration differences between carriers and noncarriers at the late postprandial time points on the other (Fig. 2).

To validate, by mathematical means, the differences in postprandial TG kinetics depicted in Fig. 2, TG curves were scrutinized by repeated measures analysis of variance (33), which permits the effects of the mutation on mean postprandial TG levels and time trends to be dissected. Carriage of the mutant gene clearly elevated the average TG concentration ($P = 0.014$) but only marginally affected the TG time trend ($P = 0.055$). A time trend effect, such as accumulation of TG-rich particles proximal to the partial block in the carriers' lipolytic cascade, however, could easily have been obscured by the simultaneous presence of TG-rich lipoproteins of different origins (intestinal and hepatic) and at different stages along the lipolytic cascade. We therefore focused on lipoproteins of intestinal origin only (by measuring RP instead of TGs) and ultracentri-

Table II. Activities of Lipoprotein-modifying enzymes

	Carriers (n = 8)	Noncarriers (n = 8)	P value	Corrected P value
LPL* (*mU/ml*)	130.8 (37.6)	348.1 (101.0)	<0.001	<0.001
HL (*mU/ml*)	354.4 (181.1)	276.9 (97.3)	0.304	0.522
CETP (*nmol/h · ml*)	130.9 (28.5)	111.4 (31.4)	0.215	0.384

Results are means (SD) as calculated from untransformed data. LPL and HL activities are in milliunits per milliliter of postheparin plasma, with 1 mU corresponding to 1 nmol fatty acids released per minute from the respective substrate emulsion; CETP activity is in nanomoles cholesteryl ester transferred from LDL to HDL per hour per milliliter of postabsorptive plasma. P values were obtained by analysis of variance, using untransformed data unless indicated otherwise; corrected P values were obtained by analysis of variance including age as covariate. * Transformation used: reciprocal of the square root.

Table III. Fasting Lipid and Apolipoprotein Levels

	Carriers (n = 8)	Noncarriers (n = 8)	P value	Corrected P value
	mg/dl			
Cholesterol*	231.9 (61.0)	179.8 (19.3)	0.038	0.190
Triglycerides	120.9 (44.8)	83.6 (27.4)	0.065	0.320
LDL-chol*	115.2 (36.9)	100.8 (15.7)	0.523	0.626
HDL-chol	56.8 (16.6)	65.3 (8.9)	0.223	0.551
HDL_2-chol*	11.5 (2.6)	19.2 (5.8)	0.005	0.022
HDL_3-chol*	45.3 (15.2)	46.1 (6.9)	0.660	0.905
Apo A-I	134.1 (14.0)	139.7 (13.1)	0.416	0.569
Apo A-II	48.6 (6.8)	44.5 (8.5)	0.306	0.619
Apo B*	103.6 (35.2)	75.8 (13.2)	0.077	0.329

Results are means (SD) as calculated from untransformed data. P values were obtained by analysis of variance, using untransformed data unless indicated otherwise; corrected P values were obtained by analysis of variance including age as covariate. * Transformation used: logarithm.

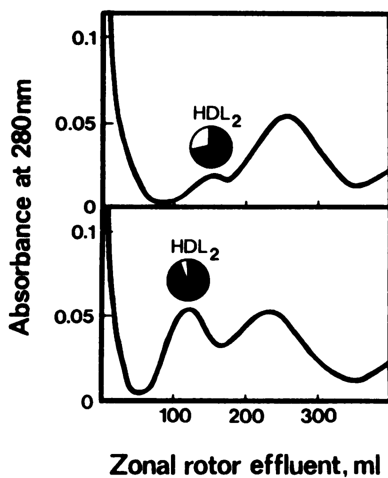

Figure 1. Representative zonal ultracentrifugal analyses of HDL subfractions in one carrier (*top panel*) and one noncarrier (*bottom panel*). The volume fraction at the center of the Beckman Ti-14 rotor contains VLDL, IDL, and LDL. HDL_2 and HDL_3 represent the peaks spanning the elution volumes from 80 to 170 ml and from 180 to 330 ml, respectively. Material eluting past 330 ml consists of the residual plasma proteins. The core compositions of HDL_2 are schematically presented in sector diagrams, with the black and white sectors denoting the weight-percentages of cholesteryl esters and TGs, respectively.

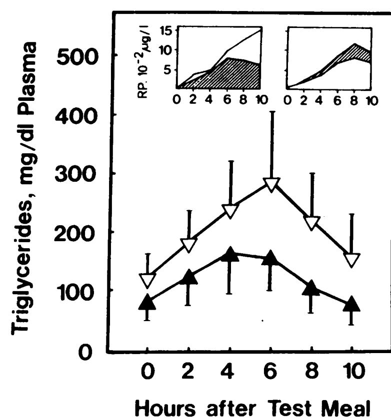

Figure 2. Postprandial triglyceride kinetics in carriers (▽) and noncarriers (▲). Bars denote one SD. (*Left inset*) Chylomicron RP kinetics in carriers (*open areas*) and noncarriers (*shaded areas*). (*Right inset*) Remnant RP kinetics in carriers (*open areas*) and noncarriers (*shaded areas*).

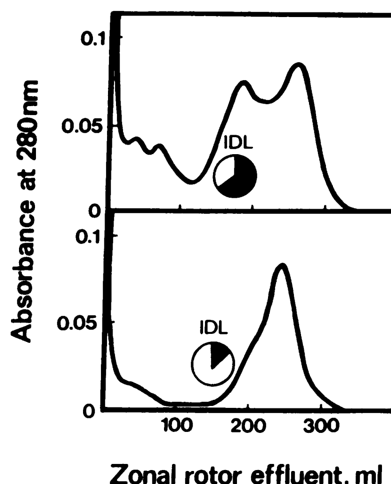

Figure 3. Representative zonal ultracentrifugal analyses of the lipoproteins with density < 1.063 g/ml in one carrier (*top panel*) and one noncarrier (*bottom panel*). VLDL elute in the first 100 ml of the Beckman Ti-14 rotor, followed by IDL (120–190 ml) and LDL (220–320 ml). The core compositions of IDL are schematically presented in sector diagrams, with the black and white sectors denoting the weight-percentages of cholesteryl esters and TGs, respectively.

fugally divided the lipolytic cascade into two compartments, one of particles with flotation rate (S_f) > 1,000 ("chylomicrons") and one of particles with S_f < 1,000 ("remnants"). In the chylomicron compartment (Fig. 2, *left inset*), RP concentrations rose until 10 h postprandially in carriers, while in noncarriers they passed through a maximum at 6 h and declined thereafter. Accordingly, carriage of the mutation not only raised the average chylomicron concentration over 10 h ($P = 0.009$) but also altered the chylomicron time trend ($P = 0.001$). In the remnant compartment (Fig. 2, *right inset*), RP levels were higher in noncarriers than in carriers throughout the postprandial period, suggesting that carriers converted chylomicrons to remnants at reduced rates. The overall difference in average remnant concentrations, however, did not show statistical significance ($P = 0.151$), and there was also no difference for carriers and noncarriers in remnant time trends ($P = 0.872$). The retinyl palmitate experiments are thus consistent with the view that it is the initial step of chylomicron metabolism which critically depends on LPL action (28, 35).

Altered distribution of cholesterol among plasma lipoproteins as a consequence of impaired TG tolerance. In noncarriers, > 90% of plasma cholesterol was associated with LDL plus HDL in the postabsorptive state, leaving a very small difference of < 10% with total cholesterol; HDL-cholesterol alone accounted for 36% of total plasma cholesterol (Table III). In carriers, the plasma cholesterol pool was distributed over lipoprotein classes very differently. First, a far smaller fraction of plasma cholesterol—only 24%—was associated with HDL. Second, the difference between total plasma cholesterol and LDL plus HDL-cholesterol was much larger, amounting to one fourth of the plasma pool (Table III). This gap indicated that in carriers a large proportion of cholesterol was present in lipoproteins of density lower than that of LDL. To locate this cholesterol unaccounted for by LDL and HDL, the lipoproteins with density < 1.063 g/ml were characterized for distribution and composition.

Typical examples of distribution analyses in carriers and noncarriers, performed by zonal ultracentrifugation of postabsorptive plasma, are contrasted in Fig. 3. In noncarriers (Fig. 3, *bottom panel*), the density < 1.063 g/ml fraction was resolved into the two typical major lipoprotein bands (18, 19), VLDL at the center of the rotor and LDL with a peak effluent volume of 240 ml. In carriers (Fig. 3, *top panel*), the LDL peak banded at somewhat higher density (260 ml), and additional lipoprotein peaks were always present. The most prominent of these peaks eluted in the IDL effluent volume (18, 36) and contained on the average 87.0 mg of IDL material/dl plasma (range 10.1–243.2 mg/dl). The cholesterol associated with this IDL amounted to one half of the cholesterol gap, i.e., the cholesterol unaccounted for by LDL plus HDL. In noncarriers, by contrast, the lipoprotein content of the respective rotor fraction was always < 10 mg/dl plasma (range 1.1–9.8 mg/dl). The IDL species isolated in abundance from carriers and that detected in only minute amounts in noncarriers differed also with respect to their composition (Fig. 3 and Table IV). While IDL from either group of individuals contained the same weight-percentage of protein—which defined its IDL density and clearly distinguished it from LDL—IDL from carriers was enriched with unesterified cholesterol at the expense of phospholipids, and to a much larger degree with cholesteryl esters at the expense of TGs (Table IV).

Cholesteryl ester-enriched IDL in abundance were a particularly striking characteristic of carriers, but by no means the

Table IV. Plasma Level and Weight-Percentage Composition of IDL

	Carriers (n = 8)	Noncarriers (n = 8)	P value	Corrected P value
IDL level* (*mg/dl*)	87.0 (80.5)	3.9 (2.9)	<0.001	<0.001
IDL protein (%)	13.8 (2.2)	13.4 (4.1)	0.790	0.538
Phospholipid (%)	18.7 (4.4)	23.2 (3.3)	0.035	0.135
Unesterified cholesterol (%)	10.0 (1.1)	6.3 (2.2)	0.001	0.006
Cholesteryl esters (%)	34.6 (9.2)	14.6 (5.2)	<0.001	0.001
Triglycerides (%)	22.9 (10.2)	42.5 (6.4)	<0.001	0.001

Unesterified cholesterol (387 mol wt); cholesteryl esters (651 mol wt). Results are means (SD) as calculated from untransformed data. The IDL level is in milligrams per deciliter; all other variables are in percent of the IDL mass. P values were obtained by analysis of variance, using untransformed data unless indicated otherwise; corrected P values were obtained by analysis of variance including age as covariate. * Transformation used: logarithm.

Table V. Core Composition of Postabsorptive Lipoproteins

	Carriers (n = 8)	Noncarriers (n = 8)	P value	Corrected P value
Cholesteryl esters in VLDL	19.9 (4.5)	12.0 (5.4)	0.007	0.015
Cholesteryl esters in IDL	60.5 (16.0)	25.6 (8.9)	<0.001	<0.001
Triglycerides in LDL	16.6 (7.5)	14.3 (3.3)	0.845	0.903
Triglycerides in HDL_2*	21.6 (7.4)	12.0 (5.7)	0.006	0.022
Triglycerides in HDL_3*	18.6 (8.5)	11.2 (5.1)	0.035	0.037

Results are means (SD) as calculated from untransformed data. Lipoprotein core masses were computed as the sums of cholesteryl esters and triglycerides. For triglyceride-rich lipoproteins (VLDL and IDL), the weight-percentages of cholesteryl esters in the respective lipoprotein's core are given. For cholesteryl ester rich-lipoproteins (LDL, HDL_2, and HDL_3), the weight-percentages of triglycerides in the respective lipoprotein's core are given. P values were obtained by analysis of variance, using untransformed data unless indicated otherwise; corrected P values were obtained by analysis of variance including age as covariate. * Transformation used: logarithm.

only one; other distinguishing features included cholesteryl ester-enriched VLDL and TG-enriched HDL_2 and HDL_3 (Fig. 1 and Table V). Also, seven of the eight carriers displayed what has been classified LDL size pattern B (37), i.e., an LDL particle distribution skewed towards small size, with a size mode < 25.5 nm. All noncarriers, in contrast, exhibited LDL size pattern A (37), i.e., an LDL particle distribution skewed towards larger size, with a size mode > 25.5 nm. The one carrier who, by expressing LDL size pattern A, broke the strict association between carrier status and LDL size was an outlier with respect to other characteristics also: of all carriers, he showed the lowest IDL concentration (10.1 mg/dl), the highest HDL_2 cholesterol level (14.2 mg/dl), and the lowest postprandial lipemia (419 mg/dl · 10 h). Thus, in this particular carrier with LDL of unexpectedly large size, the other distinguishing features were also atypical, suggesting that small-sized LDL were not a coincidental characteristic of carriers but rather a genuine component of their typical phenotype, which for some reason was not fully expressed in this individual.

Syndrome of impaired TG tolerance. As a group, carriers were distinguished from the group of the noncarriers by reduced LPL activity (Table II), lower HDL_2-cholesterol (Fig. 1 and Table III), pronounced postprandial lipemia (Fig. 2), high abundance of IDL (Fig. 3 and Table IV), enrichment of VLDL and IDL with cholesteryl esters (Tables IV and V), enrichment of HDL_2 and HDL_3 with TGs (Fig. 1 and Table V), and small size of LDL. These phenotypic characteristics were expressed in a continuous rather than a discrete fashion: the general theme held for every individual, irrespective of whether a carrier or not; the lower his or her LPL activity was, the larger was the magnitude of lipemia, the higher was the TG content of HDL_2, the lower was the HDL_2-cholesterol level, the higher was the cholesteryl ester content of VLDL and IDL, the higher was the IDL concentration, and the smaller was the size mode of LDL (Table VI). The fact that eight different phenotypic variables varied so widely between subjects but were associated so closely with any one subject suggests that all these variables figured as either determinants (in case of LPL) or dependents (in case of all other variables) of an individual's TG tolerance. Hence our proposal to call the carriers' phenotype the syndrome of impaired TG tolerance.

One could, however, still argue the remote possibility that the cause for this phenotype was not impaired TG tolerance due to carriage of the mutant LPL gene, but rather a second abnormality with which it was compounded fortuitously. We therefore tested for potential links with apoE isoforms, HL activity, and CETP activity, because these variables can also be associated with one or more of the carriers' stigmata: apoE isoforms with changes in concentration and cholesteryl ester enrichment of VLDL and IDL (36, 38) and with changes in the magnitude of lipemia (39), HL activity with changes in the levels of HDL_2 and IDL (40–42), and CETP activity with

Table VI. Correlation Matrix of LPL Activity, Magnitude of Lipemia, and Concentration, Core Composition, and Size of Lipoproteins

	LPL*	Lipemia‡	HDL_2-cholesterol‡	Triglycerides in HDL_2‡	Cholesteryl esters in VLDL	IDL level‡	Cholesteryl esters in IDL	LDL Size‡
LPL*	1.000	−0.631‖	0.557§	−0.783**	−0.521§	−0.745**	−0.632‖	0.561§
Lipemia‡		1.000	−0.587§	0.668¶	0.225	0.551§	0.461§	−0.690¶
HDL_2-cholesterol‡			1.000	−0.764**	−0.534§	−0.573§	−0.554§	0.447
Triglycerides in HDL_2‡				1.000	0.554§	0.756**	0.672¶	−0.568§
Cholesteryl esters in VLDL					1.000	0.629‖	0.804**	−0.424
IDL level‡						1.000	0.865**	−0.759**
Cholesteryl esters in IDL							1.00	−0.807**
LDL size‡								1.000

Triglycerides in HDL_2 is the weight-percentage of triglycerides in the HDL_2 core; cholesteryl esters in VLDL and cholesteryl esters in IDL are the weight-percentages of cholesteryl esters in the VLDL and the IDL core, respectively. Carriers and noncarriers were pooled for calculation of second-order partial correlation coefficients, controlling for age and sex. For every bivariate correlation, the adequacy of a combined analysis of carriers and non-carriers was examined with a battery of graphical techniques, including scatterplots identifying carriers and noncarriers, normal probability plots of the Studentized residuals, and plots of the Studentized residuals against the predicted values (32). * Transformation used: reciprocal of the square root. ‡ Transformation used: logarithm. Two-tailed significance levels: § $P < 0.05$; ‖ $P < 0.01$; ¶ $P < 0.005$; ** $P < 0.001$.

changes in the core composition of all lipoproteins (43–46). Each of these tests gave a negative result. The frequencies of apoE isoforms were identical in carriers and noncarriers; 12 study subjects, six carriers and six noncarriers, possessed the apoE3/3 phenotype, and one carrier and one noncarrier each the apoE3/2 and the apoE4/3 phenotype, respectively. The activities of neither HL nor CETP differed significantly between carriers and noncarriers (Table II). More importantly, HL activity was related to none of the variables shown in Table VI, and CETP activity only to the cholesteryl ester content of VLDL ($r = 0.6861, P < 0.01$) and IDL ($r = 0.5188, P < 0.05$). Thus, of the three lipoprotein-modifying candidate enzymes LPL, HL, and CETP, only the variation in LPL activity could explain all aspects of the observed phenotypic variation. However, our analysis quite reasonably concedes that some signs of the syndrome may be modulated by factors additional to TG tolerance; for instance, the core composition of TG-rich lipoproteins by CETP.

Discussion

The present study delineates the effect of a defective LPL gene in single dose on TG metabolism under relaxation and strain, and tracks down its impact on concentration, composition, and size of the major plasma lipoprotein classes. In a previous investigation (16), heterozygous carriers of the mutation at codon 188 of the LPL gene presented with fasting hypertriglyceridemia only when obese, hyperinsulinemic, mildly hyperglycemic, or hypertensive, or when receiving estrogens, thiazide diuretics, or β-adrenergic antagonists. None of the carriers in the present study was compromised by any of these aggravating factors; accordingly, differences between affected and unaffected family members were null or minor with respect to the routinely measured lipids, TGs, cholesterol, and HDL-cholesterol, in the postabsorptive state. Elaborate analyses, however, revealed distinct deviations from the normal phenotype even in these normotriglyceridemic carriers: TG-rich lipoproteins accumulated in the postprandial state, and as a typical consequence (24, 34), the carriers' HDL_2 particle pool was depleted, with the remaining HDL_2 consisting of TG-enriched particles. The TG-rich lipoproteins, in turn, were enriched in cholesteryl esters, IDL occurred in abundance, and the Stokes diameter of LDL was reduced. Collectively, these deviations constitute the components of what we propose to call the syndrome of impaired TG tolerance.

Although the syndrome of impaired TG tolerance appears to clearly segregate with the mutant allele, some cautionary remarks may be appropriate. First, manifestation of hypertriglyceridemia in heterozygous LPL deficiency has been demonstrated to be age-modulated (16), and a perfect match of our study subjects with respect to age was not possible (Table I). However, a substantial bias of our results due to the higher average age of carriers can be ruled out by two lines of reasoning: (*a*) All statistical analyses included age as a covariate. (*b*) While age may be a major factor in the expression of gross fasting hypertriglyceridemia, most likely by adding an increased rate of VLDL production to the carriers' decreased rate of VLDL degradation (16), the same need not be true for the more subtle derangement of impaired TG tolerance. Indeed, the present study shows that an exogenous fat load can unveil impaired TG tolerance before age-related overproduction of VLDL provides sufficient endogenous challenge to produce fasting hypertriglyceridemia.

A second aspect deserving some consideration is that our study subjects originated from two different families. Because the numbers of individuals recruited from either family were small and unequal (six and two carriers and five and three noncarriers, respectively), quantitative comparisons of carriers and noncarriers within one family, or of carriers and noncarriers between the two families, were not feasible. However, we have qualitatively ascertained that pooling members of the two families for analysis was adequate. When our 16 study subjects were ranked with respect to the variables of interest and the data displayed graphically, differences were conspicuous only between carriers and noncarriers, but not between members of the two families.

How, then, does impaired TG tolerance translate into the multiple phenotypic characteristics of the syndrome? We favor a scenario according to which the two major lipids transported in plasma, cholesteryl esters and TG, remain essentially sequestered in two distinct lipoprotein families, one of TG-rich particles (chylomicrons and VLDL) and one of cholesteryl ester-rich particles (LDL and HDL), when TG metabolism operates efficiently. This fairly strict separation of the two lipid species, however, tends to break down when TG tolerance is impaired because accumulation of triglycerides in plasma drives core lipid exchanges between lipoproteins (43–48). TGs are transferred from TG-rich lipoproteins to LDL and HDL; cholesteryl esters, in turn, are withdrawn from LDL and HDL and incorporated into TG-rich lipoproteins. With this altered core composition of all lipoproteins, the distinction between former TG-rich and former cholesteryl ester-rich particles becomes less clear (Table V), and the cholesterol gap (the difference between total plasma cholesterol and LDL plus HDL-cholesterol) widens. Replacement of lipase-susceptible TGs by lipase-resistant cholesteryl esters in chylomicrons and VLDL impedes their undisturbed passage through the lipolytic cascade (49), and abnormally cholesteryl ester-rich intermediates such as IDL accumulate, also a distinctive feature of impaired TG tolerance (Fig. 3). The reciprocal crossing-over of TGs from TG-rich lipoproteins to LDL and HDL, in turn, reduces the proportion of nondegradable cholesteryl esters in these particles' cores and, thus, opens the way for their size reduction through hydrolysis of the transferred triglycerides by LPL and/or HL (50, 51). Shunting of TGs into former cholesteryl ester-rich lipoproteins therefore explains the remaining three characteristics of the syndrome of impaired TG tolerance: the low HDL_2-cholesterol level (Table III), the preponderance of the small HDL subfraction HDL_3 (Fig. 1), and the reduced size mode of LDL.

Small-sized LDL are the hallmark of a lipoprotein trait which exhibits remarkable similarities with the syndrome of impaired TG tolerance, but was introduced in a different context (37, 52). Starting from the observation that two distinct LDL subclass distributions exist in the population—labeled pattern A and B, depending on the predominance of larger and smaller LDL, respectively (37)—lipid and lipoprotein correlates of LDL size were sought and detected: small size of LDL, i.e., pattern B, was found frequently with elevated levels of TGs, VLDL, and IDL, and with depressed levels of HDL-cholesterol and HDL_2 (52). As would be expected from this clustering of cardiovascular risk factors, LDL pattern B and its associated abnormalities were found to confer threefold higher

odds of suffering a myocardial infarction than pattern A (37), hence its connotation as the "atherogenic lipoprotein phenotype" (52). Complex segregation analyses in normolipidemic (53) and combined hyperlipidemic (54) kindreds suggested that the size of LDL is controlled by a single major locus designated *ATHS* (for atherosclerosis susceptibility), with dominance of the postulated B allele but incomplete penetrance in young males and premenopausal females. According to a linkage analysis based on this simple Mendelian model, the *ATHS* gene maps to the short arm of chromosome 19, near or at the LDL receptor locus and in close proximity to the insulin receptor locus (55).

The present investigation took the reverse approach (56). Starting from an exactly defined single-gene defect, we quantified and characterized TG tolerance, lipids, and lipoproteins and linked these characteristics to carriage of the mutation. The carriers' phenotype encompassed a characteristic metabolic constellation, i.e., that of pronounced postprandial lipemia with low levels of TG-enriched HDL_2 (24, 34, 50). In addition, all criteria of the atherogenic lipoprotein phenotype were recovered in carriers, including the *sine qua non* of this trait, LDL of small size. These close phenotypic similarities suggest that the metabolic setting underlying the atherogenic lipoprotein phenotype is also impaired TG tolerance, of whatever molecular cause. The existence of a single-gene defect causing impaired TG tolerance thus appears to unite, by serendipity, two avenues of research by demonstrating that carriers display all the signs hitherto individually linked either to pronounced lipemia or to LDL pattern B. In this way, our study may provide not only a detailed description of heterozygous LPL deficiency but, more generally, of impaired TG tolerance, specifying the first condition as a paradigm for the second.

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