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Lipoprotein abnormalities in compound heterozygous lipoprotein lipase deficiency after treatment with a low-fat diet and orlistat

Piers Blackett, MB, ChB, FNLA*, Jeanie Tryggestad, MD, Sowmya Krishnan, MD, Shibo Li, PhD, Weihong Xu, PhD, Petar Alaupovic, PhD, Carmen Quiroga, Kenneth Copeland, MD

Diabetes and Endocrinology Section, Department of Pediatrics, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA (Drs. Blackett, Tryggestad, Krishnan, and Copeland); Medical Genetics, Department of Pediatrics, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA (Drs. Li and Xu); and Lipid and Lipoprotein Laboratory, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA (Dr. Alaupovic and Ms. Quiroga)

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BACKGROUND: The treatment of familial hyperchylomicronemia presenting in early childhood with episodes of pancreatitis has been ineffective, and affected patients remain at risk for pancreatitis.

OBJECTIVE: To report on the effect of orlistat in siblings with severe inherited hyperchylomicronemia and to assess posttreatment lipoprotein concentrations and composition.

METHODS: Serial observations of plasma lipid levels and hospitalizations after treatment with orlistat and lipoprotein studies on a single fasting posttreatment sample.

RESULTS: The affected siblings inherited a lipoprotein lipase gene mutation from each of their parents: a novel mutation from their father (c.542G > C, p.G181R) and a known missense mutation from their mother (c.644G > A, p.G215E). When the boy presented to us at age 9 years of age and his sister at age 7 years, we found that addition of orlistat, a pancreatic lipase inhibitor, at a dose of 120 mg given before three low-fat meals a day was effective in reducing episodes of pancreatitis in the boy and in maintaining the triglyceride at lower levels in both children. During treatment, the children were observed to have elevations in apolipoprotein (apo)B, low-density lipoprotein particle concentration, abnormal apoB-containing subclasses, and deficiencies in apoA-I and apoA-II-containing lipoproteins, changes consistent with continuing increased cardiovascular risk.

CONCLUSION: The data support the need for more effective long-term treatments that not only prevent pancreatitis but also offset cardiovascular risk. Orlistat can be considered effective in augmenting the effect of a low-fat diet and reducing risk for pancreatitis.

Familial chylomicronemia, although rare (affecting 1 in 1,000,000),[1,2] presents in patients as severe lipemia associated with triglyceride levels greater than 2000 mg/dL and recurrent pancreatitis even with stringent dietary fat restriction.[3] Conventional triglyceride-lowering agents that down-

* Corresponding author.
E-mail address: piersblackett@att.net

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regulate very-low-density lipoprotein (VLDL) production and enhance lipoprotein lipase such as fibrates and omega-3 fatty acids usually have been ineffective because VLDL is seldom elevated and lipoprotein lipase remains defective.[2] Recent trials of incorporating the lipoprotein lipase gene into muscle using the adeno-associated viral vector-1 coupled to a gain-of-function lipoprotein lipase gene variant (AAV1-LPL^{S447X}) have been encouraging. Initial results showed that a single administration of multiple intramuscular injections given under spinal anesthesia transiently lowered triglyceride levels for 5 months[4]; however, postprandial chylomicron clearance was improved by 93% at 14 weeks.[5] This therapy is considered to be in a developmental phase, only achieving partial success,[4,6] and the long-term effectiveness and potential for the development of this treatment method is unclear.

We present evidence that hyperchylomicronemia can be improved and the risk for pancreatitis can be reduced by the administration of orlistat (Xenical; Genentech, South San Francisco, CA), a pancreatic lipase inhibitor given before meals in affected siblings on a fat-restricted diet. In addition to documenting reduction in fasting triglyceride, we also studied the status of the remaining lipoprotein profile to determine whether lipoproteins with atherosclerotic characteristics are present in the affected children and their carrier parents.

Subjects

Case 1

A 12-year-old boy first presented at 3 months of age. His plasma was found to be lipemic during a preoperative evaluation for perirectal abscess. Subsequently, he experienced recurrent abdominal pain, and triglyceride levels increased to >13,000 mg/dL in the first year. Fat was restricted to less than 10 g per day, and medium-chain triglycerides were added.

At age 2 years 10 months, the patient's triglyceride levels were 1264 mg/dL, cholesterol 178 mg/dl, and high-density lipoprotein cholesterol (HDL-C) 11 mg/dL. Apolipoprotein (Apo)C-II was reported to be present. Amylase was 68 (25–125) and lipase 20 U/L (10–140). He first developed pancreatitis at age 5 years and had eruptive xanthomata. He presented to our clinic at the age of 8 years and was admitted to the hospital with mild acute pancreatitis and a history of 14 previous episodes of pancreatitis requiring hospital admissions. Triglyceride levels were 2670 mg/dL, cholesterol 1370 mg/dL, and HDL-C 10 mg/dL. Magnetic resonance imaging showed that the head of the pancreas appeared large and edematous; peripancreatic fluid tracked to the right of the abdomen, a short segment of regional colon was thickened, and there were lymph nodes seen in the right upper and lower quadrants consistent with inflammation.

After restriction of all oral intake and treatment with intravenous fluids, he was placed on restricted fat intake to less than 20 g per day with supplemental vitamins A, D, E, and K; vitamin C; beta-carotene; and orlistat 120 mg before meals. During the following year, he had only one episode of pancreatitis and has been symptom-free over the past 3 years, except for occasional oily stool leakage and flatulence. Lipase and amylase activities have remained normal. The fasting triglyceride levels under home conditions has ranged from 500 to 600 mg/dL over 31 months (Table 1), but the fasting triglyceride was increased on the day of the study (at age 12 years) after they had stayed at a nearby hotel overnight (Table 2). Body mass index (BMI) was 18.9, and there were no abnormal physical findings. He had attained Tanner stage 2 for puberty. Vitamin levels (with normal ranges) while the patient was receiving supplemental treatment were as follows: vitamin A, 26 μg/dL (18–77), vitamin E, 23 mg/dL (4.6–17.8), vitamin K, 5.02 ng/dL (0.28–1.78), and 25(OH)D, 20 ng/mL (30–100). The dose of vitamin D subsequently was increased to 800 mIU per day.

Case 2

A 10-year-old girl, the younger sister of case 1, first presented in the newborn nursery with a triglyceride level of >500 mg/dL, which increased to >15,000 mg/dL after the first month while she was breastfed. She was treated

Table 1 Lipid values in the affected siblings before and after orlistat treatment given as 120 mg before meals during 17 months (girl patient) and 31 months (boy patient)

Girl					Boy				
Months	TG, mg/dL	TC, mg/dL	TG:TC	HDL-C, mg/dL	Months	TG, mg/dL	TC, mg/dL	TG:TC	HDL-C, mg/dL
0†	2820	320	8.8	10	0*,†	2670	370	7.2	10
6	570	215	2.6	13	14	960	173	5.5	14
17	504	196	2.6	17	20	549	123	4.5	16
					31	515	144	3.6	15

HDL-C, HDL-cholesterol; TC, total cholesterol; TG, fasting triglycerides; TG:TC, triglycerides to cholesterol ratio.
*Patient had acute pancreatitis.
†Orlistat treatment commenced.

Table 2 Lipids and apolipoproteins

	Girl	Boy	Mother	Father	Normal ± SD*
Triglycerides, mg/dL					
Total	2180†	1225†	136	247†	84.8 ± 42.8
Chylomicron	1337†	457†			None
Chylomicron-free	803†	768†			84.8 ± 42.8
Cholesterol, mg/dL					
Total	250†	185	195	206	168.6 ± 33.2
Chylomicron	120	102			None
Chylomicron-free	130	83			168.6 ± 33.2
HDL-C, mg/dL	38	17	65	42	53.3 ± 15.2
ApoA-I, mg/dL					
Total	197†	131	143	126	132.3 ± 17.7
Chylomicron	90.9†	46.5†			None
Chylomicron-free	106.1	84.5			132.3 ± 17.7
ApoA-II, mg/dL					
Total	105.2†	129.6†	45.8	41.7	39.8 ± 8.0
Chylomicron	76.7†	101.6†			None
Chylomicron-free	28.5	28			39.8 ± 8.0
ApoB, mg/dL					
Total	403.5†	266.5†	81.3	97.1†	87.6 ± 12.7
Chylomicron	270.5†	157.5†			None
Chylomicron-free	133†	106.3†			87.6 ± 12.7
ApoC-III, mg/dL					
Total	126†	79†	10.6	12.4	9.5 ± 2.6
Chylomicron	106.7†	69.5†			None
Chylomicron-free	19.3†	9.5			9.5 ± 2.6
C-III HP (chylomicron-free)‡	6.4	7.9	3.1	6.7	3.4 ± 1.7
C-III HS (chylomicron-free)	2.7	8.8	6.8	5.0	6.1 ± 2.2
C-III ratio	0.42†	1.11†	2.19	0.75†	2.0 ± 0.9
ApoE, mg/dL					
Total	36.5†	53†	3.6	6.5	5.4 ± 1.2
Chylomicron	32.5†	49.2†			None
Chylomicron-free	4	3.8			5.4 ± 1.2

Apo, apolipoprotein; HDL-C, HDL-cholesterol.
*Normal values were obtained from an adult control population (n = 60).
†Abnormal values.
‡Heparin-manganese was used to separate apoB-containing particles in the precipitate (HP) from apoA-I-containing particles in the supernate (HS).

with restricted-fat diet and a formula containing medium-chain triglycerides. At 6 years and 11 months, triglyceride levels were 2820 mg/dL, cholesterol 320 mg/dL, HDL-C 10 mg/dL, and lipase 23 U/L without symptoms. She was treated with orlistat 120 mg before meals and remains symptom-free except for one admission to the hospital for abdominal pain associated with normal lipase and amylase values and a benign course. Follow-up fasting triglyceride levels performed under home conditions have been below 600 mg/dL at two follow-up visits during a 17-month period (Table 1), but at the time of the study, after an overnight stay near the hospital, her triglyceride level was 2180 mg/dL (Table 2). Her BMI was 20.7, and there were no abnormal physical findings. She had attained Tanner stage 2 for puberty. Vitamin levels for the patient's supplemental treatment were as follows: vitamin A, 28 μg/dL (18–77); vitamin E, 26.5 mg/dL (4.6–17.8); vitamin K, 3.55 ng/mL 0.28–1.78); and 25(OH)D, 27 ng/mL (30–100). As with her brother, the dose of vitamin D was increased to 800 mIU/day.

Family history

The mother's sister developed pancreatitis associated with hypertriglyceridemia at the age of 40 years. The paternal grandfather and the grand-father's three siblings have moderately high triglyceride levels. Both parents have a rural lifestyle and have been otherwise healthy. The mother's BMI was 23.5 and the father's was 32.0.

Laboratory methods

A protocol for the studies was approved by the Institutional Review Board at the University of Oklahoma Health Sciences Center, and informed consent was obtained from the participating family members. The study met

requirements for exemption from investigational new drug approval as determined by the Food and Drug Administration because we were in compliance with requirements for institutional review and informed consent. Blood samples were obtained after an overnight fast for more than 12 hours. Baseline fasting pretreatment samples were obtained at clinic visits, but samples for routine clinical follow-up were conducted locally (Table 1). Samples for detailed lipoprotein studies (Tables 2, 3, and 4) were also obtained fasting after an overnight stay near the medical center in Oklahoma City.

Gene studies

Total genomic DNA was extracted from peripheral white blood cells by the use of a QuickGene Automated extractor (Autogen, Holliston, MA). mRNA was isolated from leukocytes using a QIAamp RNA Blood Mini Kit (QIAGEN, Valencia, CA). The first-strand cDNA was reverse-transcribed using a SuperScript III Reverse Transcriptase Kit (Invitrogen, Carlsbad, CA) and random primers. Polymerase chain reaction was performed using a touchdown program and the designed primers corresponded to the coding region and the flanking areas of apoC-II and lipoprotein lipase (LPL) genes. The dideoxy nucleoside triphosphate terminator reaction (ddNTP) was performed with an ABI BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems). Sequencing data were collected by the ABI 3130*xl* genetic analyzer (Applied Biosystems) for *APOC2* and *LPL*.

Lipids and apolipoproteins

An Abbott VP-Super System automatic analyzer and commercial reagents were used to determine levels of cholesterol (Boehringer, Mannheim, Federal German Republic) and triglyceride (Miles Inc., Tarytown, NJ) by enzymatic methodology. HDL-C was measured following the heparin-manganese precipitation procedure of the Lipid Research Clinics program, and low-density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald formula. ApoA-I, apoB, and apoC-III were determined by standardized immunoturbidimetric methods using polyclonal antibodies.[7] ApoC-II and apoE were measured by electroimmunoassay. Assays on plasma from the affected boy and girl were done with and without the chylomicrons. Chylomicrons were removed after the sample had remained standing for more than 12 hours to obtain chylomicron-free plasma. The assays were conducted at the Oklahoma Medical Research Foundation in the laboratory of Petar Alaupovic, PhD.

Apolipoprotein-based lipoprotein subclasses (LpB, LpB:C, LpB:E + LpB:C:E, and LpA-II:B:C:D:E) were determined by sequential immune precipitations.[8] ApoB-containing subclasses were performed in three separate steps on the basis of sequential immunoprecipitation of whole plasma by polyclonal antisera to apoA-II, apoE, and apoC-III, respectively. To simplify the procedure, the LpB:E and LpB:C:E subclasses were measured together. The preparation of antisera was carried out according to a previously described procedure.[9] Lipoprotein subclass sizes and concentrations were determined by nuclear magnetic resonance by Liposcience Incorporated.[10]

Results

LPL gene mutations

No variants were detected in the apoC-II gene (*APOC2*). Compound heterozygous mutations c.542G > C (p. G181R) and c.644G > A(p.G215E) were found in exon 5 of the lipoprotein lipase gene of both children. The parental samples were sequenced to determine the mutations in *cis* or *trans*. G181R observed in the father, is a novel missense mutation; and the missense mutation, G215E observed in the mother has been described previously (Human Gene Mutation Database). Both mutations also were confirmed at the mRNA level proving compound heterozygous inheritance of the two alleles in both children.

Lipids and apolipoproteins

The triglyceride-lowering effect is shown over 17 months in our female patient and over 31 months in our male patient (Table 1). Fasting triglyceride values during treatment with orlistat (120 mg orally before meals) decreased from values greater than 2000 mg/dL to less than

Table 3 Lipoprotein subclasses according to apolipoprotein content

	Girl (chylomicron-free)	Boy (chylomicron-free)	Mother	Father	Normal, mean ± SD*
LpA-II:B:C:D:E	25.3†	30.2†	7.3	14.7	11.5 ± 65.9
LpB:E + LpB:C:E	14.3	12.5	14.6	14.5	11.1 ± 4.0
LpB:C	10.4	30.7†	4.8	9.3	8.1 ± 3.7
LpB	56.3	59.6	54.6	58.6	57.1 ± 7.3
LpA-I	19.6†	23.8†	34	27.6†	34.5 ± 5.3
LpA-I:A-II	64.9†	82.3†	109	98.4	97.8 ± 13.7

Lp, lipoprotein.
*Normal values were obtained from a local adult control population (n = 60, ages 18–72, mean 40 ± 12.9 years).
†Abnormal values.

Table 4 NMR lipoprotein profile performed on whole plasma

	Girl	Boy	Mother	Father	Normal,* Mean ± SD
VLDL and chylomicron concentrations, nmol/L					
VLDLCP	121.5†	65.4†	27.4†	82.1†	77.3 ± 65.5
VLCP	118.2†	44.7†	4.5†	12.4†	3.0 ± 7.7
VMP	0†	0†	15.7†	58.1†	46.7 ± 52.2
VSP	3.3†	20.7†	7.2†	15.7†	28.1 ± 26
LDL particle concentrations, nmol/L					
LDLP	651	621	1486	1749	1514 ± 489
IDLP	80†	187†	27	84†	27 ± 44
LLP	0	0	652	499	422 ± 279
LSP	571	434	806	1166	1065 ± 564
HDL particle concentrations, mmol/L					
HDLP	20.4†	20.7†	51.8†	33.5	30.5 ± 7.6
HLP	1.5†	0.4†	6.8	1.6†	7.0 ± 4.6
HMP	1.1	4.2	27.2†	3.1	2.7 ± 3.7
HSP	17.8	16.1	17.8	28.8	20.8 ± 5.7
Mean particle sizes, nm					
VZ	84.3†	83.6†	55.7	59.8	53.6 ± 15.0
LZ	19.6	19.7	21.2	20.2	20.7 ± 0.9
HZ	8.3	8.6	8.9	8.1	8.9 ± 0.4

Lipoproteins: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins.
Lipoprotein Particle Concentrations: VLDLCP, total VLDL + chylomicrons; VLCP, large VLDL; VMP, medium VLDL; VSP, small VLDL; LDLP, total LDL; IDLP, intermediate density lipoproteins; LLP, large LDL; LSP, small LDL; HDLP, total HDL; HLP, large HDL; HMP, medium HDL; HSP, small HDL.
Lipoprotein Particle Sizes: VZ, VLDL; LZ, LDL; HZ, HDL.
*Normal values were obtained from a control population (age 10-94 years, median 58 years).
†Abnormal values.

600 mg/dL and treatment was associated with decreases in total cholesterol, and a slight increase in HDL-C, which remained very low. During this time the triglyceride to cholesterol ratio decreased, consistent with a decrease in triglyceride-rich chylomicrons containing relatively more triglyceride than cholesterol compared to VLDL.

The children's apoC-II values were lower than the parents. Case 1: 1.0 mg/dL, case 2: 1.1 mg/dL, mother: 2.1 mg/dL, father: 1.7 mg/dL (normal average mean: 4 mg/dL). The low values suggested that partial deficiency could cause the high triglyceride levels. Gene sequencing showed a normal *APOC2* gene. Lipid and apolipoprotein values (Table 2), apolipoprotein-based lipoprotein subclasses (Table 3), and nuclear magnetic resonance lipoprotein profile data (Table 4) are presented and the abnormal results are listed in bold.

Discussion

Supplementing a low-fat diet with premeal orlistat for more than a year in siblings aged 6 and 8 years with severe familial chylomicronemia caused by compound heterozygous LPL deficiency, resulted in triglyceride-lowering and prevention of potentially fatal pancreatitis over more than a year of observation. Because the lipoprotein lipase deficiency results in severe impairment of lipid clearance, it follows that minimizing intestinal fat absorption by inhibiting pancreatic lipase results in triglyceride lowering. Tzotzas et al[11] reported that a 34-year-old man with familial chylomicronemia who had only a trace of LPL activity in postheparin plasma, benefited from the same premeal doses of orlistat given during a 12-week period with a 33% reduction in fasting triglyceride. However, use of orlistat for this condition has not been widely recognized and has not been previously used to treat children with severe inherited lipoprotein lipase deficiency.

Although the use of orlistat has been standardly quoted in texts as being effective in treating severe hypertriglyceridemia, published information has been limited. A frequently quoted case report describes a 39-year-old nonobese Asian male with combined hyperlipidemia and an initial triglyceride of 766 mg/dL who was found to be resistant to treatment with a reduced-fat diet and gemfibrozil. The addition of orlistat resulted in 70% triglyceride lowering showing synergism, since the combination was shown to be more effective than either drug alone.[12] Although the triglyceride levels did not exceed 2000 mg/dL (22.6 mmol/L) as in cases with monogenic defects in lipoprotein lipase activity, partial deficiency could explain his resistance to gemfibrozil and subsequent response to the addition of orlistat. It is possible that compliance with dietary fat restriction may offset any further response to orlistat; however, this is not the case in our monogenic siblings, who remain on very low fat intake while on orlistat but respond with marked increases in triglyceride to small

and often-unintentional increases in fat. Alternatively noncompliance with prescribed standard reductions in fat intake could explain the resistance to orlistat, but this becomes apparent to the patient with the appearance of oily stools and flatulence caused by undigested fat.

The efficacy of treatment directed at further reducing intestinal fat in severe hypertriglyceridemia is further suggested by observations in five adults who were reported to have had a further 35% decrease in triglyceride after the addition of orlistat to their existing medications[13] supporting its wider use. Pancreatic lipase inhibition with orlistat contrasts with conventional treatments, such as fibrates, omega-3 fatty acids, and niacin, which have been used to increase plasma triglyceride clearance and decrease hepatic VLDL production,[14–16] but these latter agents are without effect in familial chylomicronemia[2] because defective chylomicron clearance by lipoprotein lipase persists and is resistant to enhancement. On the basis of the rationale that treatment with orlistat can reduce calories from fat ingestion, it has also been used for weight loss in adolescent patients who are obese,[17] providing evidence that the drug can be used at young ages with a low side-effect profile. Furthermore, previous investigators have shown antidiabetic and antiatherogenic properties of orlistat in addition to its effect on weight loss,[18] and LDL-C can be lowered by 10%.[19]

Because orlistat inhibits fat absorption by about 30% in cases with normal pancreatic lipase,[20] it is advised in the package insert that all patients take a daily multivitamin that contains vitamins A,D, E, and K.[21,22] We observed low levels of 25(OH)D, indicating vitamin D deficiency, which was corrected by increasing the dose. The incidence of vitamin D deficiency in association with orlistat therapy is more frequent than with the other fat-soluble vitamins and occurs in 12% of adults (6.6% for placebo) and in 1.4% of adolescents (1.4% for placebo); however, the required severe reduction in fat intake is more likely to cause deficiency in familial chylomicronemia than when prescribed for obese cases whose dietary restriction is less stringent.

In addition medium-chain triglycerides are recommended as a source of fat because they are directly metabolized by the liver and do not form chylomicrons[20]; however, recurrent pancreatitis in our boy patient was not prevented with diet and medium-chain triglycerides as the only additive. Gastrointestinal side-effects attributed to orlistat were minimal, possibly because the fat intake remained very low, thus minimizing the undigested fat.

Missense lipoprotein lipase mutations, G181R and G215E, located in exon 5 in each of the parents, resulted in mixed heterozygous deficiency in the affected children with confirmation at the RNA level. In general lipoprotein lipase genes cluster in exons 5 and 6,[23] and it has been proposed that the resulting mutant proteins are unable to achieve or maintain normal dimer conformation and become inactive.[24] The father carries G181R, which is close to the more commonly observed Gly188Glu mutation,[25] and has not been described previously. The maternal mutation, G215E, is located at a site determining conformation of the central catalytic domain,[26] and is rare but not novel. Although it is listed in an international database, it has not yet been described in a peer-reviewed publication. Although we have not measured lipoprotein lipase enzyme mass and activity, confirmation by genotyping associated with a severe clinical course was considered sufficient for diagnosis of compound heterozygous disease. The paternal allele caused a moderate increase in fasting triglyceride in the father, and the mother had a random postprandial triglyceride level that was greater than 2000 mg/dL although her fasting level was normal, suggesting that her triglyceride levels are susceptible to increases in the postprandial state; furthermore, her presumed heterozygous sister has a history of pancreatitis and severe hypertriglyceridemia.

Because both affected children had significant hyperchylomicronemia shown in standing plasma and in the nuclear magnetic resonance profile, it was of interest to observe increases in the apolipoprotein content of the chylomicrons. The very high apoB levels in the lipemic plasma approached normal in chylomicron-free plasma, indicating that the polyclonal apoB antibody detected an abundance of intestinally derived apoB-48–containing chylomicrons contributing to a very high total apoB. These lipoproteins, having undergone prolonged plasma retention secondary to the lipoprotein lipase deficiency, are likely to contain a combination of apoB-48 originating from the intestine as chylomicrons, and apoB-100 originating from the liver in the form of VLDL. A relatively increased intermediate-density lipoprotein (IDL) particle number is consistent with a large pool of non-HDL particles including chylomicron remnants.

Total plasma apoE and apoC-III were markedly increased in the chylomicrons, indicating that they acquire apoC-III and apoE resulting in high total levels, but the levels were normal in chylomicron-free plasma. This finding can be explained by apoC-III and apoE not being integrated with the particle at the time of secretion but transferred to the chylomicrons from other lipoproteins, including HDL.[27] However, the apoC-III ratio calculated as the proportion of apoC-III in HDL to non-HDL measured in the heparin precipitated fraction was lower than the cut-point, reflecting poor lipolysis of triglyceride-rich particles. This concept is supported by observations that apoC-III normally undergoes transfer during lipolysis proportionate to the rate of lipolysis[28] and postheparin lipolytic activity.[29] ApoE, known to have a similar bimodal distribution to apoC-III, was also highly associated with the chylomicrons in both affected children. The unusual distribution can be attributed to transfer from HDL and suggests that the normal protective function of apoE in enhancing remnant removal by LDL receptor uptake[30] is offset by being sequestered in chylomicrons.

Because apoA-I, like apoB, is considered an integral apolipoprotein, transfer among lipoproteins is minimal. It appeared normal in the boy and his parents but high in the girl before chylomicron removal, which is consistent with a proportion of apoA-I originating from the intestine as well as the liver[31] in the form of lipid-poor apoA-I. The apoA-II,

however, was high in both affected children with a significant proportion in the chylomicrons consistent with an intestinal origin or possibly excessive transfer from HDL. The HDL-C was remarkably low in the affected children, particularly in the boy, and the HDL particle concentration was low, particularly in the large-sized particle subclass suggesting poorly functional HDL in regard to cholesterol enrichment by efflux from peripheral cells. Also, interaction of cholesterol ester transport protein with the triglyceride-rich particles particularly in the postprandial state[32] is likely to be associated with increased cholesterol ester transport activity.[33] Consequently HDL enrichment with triglyceride and subsequent degradation by intact hepatic triglyceride lipase[34] probably contributes to the significant HDL-C lowering. The lower HDL-C and apoA-I in the boy can be attributed to pubertal onset and the influence of testosterone during puberty.[35]

Lipoprotein subclasses determined by serial immune-precipitations of the respective apolipoproteins[8,9] in chylomicron-free plasma, provided further characterization of the remaining particles and insight on long term risk for atherosclerosis. Because apoB-containing subclasses have variable affinities for lipoprotein lipase with LpA-II:B:C:D:E having the least,[36] the remarkably elevated LpA-II:B:C:D:E particles in chylomicron-free plasma in both affected children support their dependence on lipoprotein lipase for disposal. Resistance of triglyceride-rich lipoproteins to lipoprotein lipase has been previously described[37] and is considered to be an independent cause of hypertriglyceridemia.[38] LpB:C particles also are increased, particularly in the boy and are associated with increased cardiovascular risk supported by evidence that apoB-containing particles that also contain apoC-III are stronger predictors of atherosclerosis than LDL-C[39] or particles that contain apoB alone.[40]

On the basis of our data on the subclasses determined by apolipoprotein composition and an increase in IDL particle concentration, it appears likely that cases with familial chylomicronemia are at risk for premature cardiovascular disease as previously reported in four cases,[41] particularly in the presence of low apoA-I, HDL-C, and HDL particle concentration. Although we reduced the risk of pancreatitis by triglyceride-lowering, the HDL-C remained low as shown by routine follow-up lipid profile testing (Table 1), suggesting that cardiovascular risk was unchanged by orlistat. Other than detailed studies on a single posttreatment sample, the follow-up studies were confined to lipid profiles and LDL-C could not be derived because of the hypertriglyceridemia. However, the lipoprotein particle data derived from a single posttreatment chylomicron-free plasma sample (Tables 2 and 3) support attention to cardiovascular risk factors, lifestyle prescription and appropriate treatment when indicated.

Because heterozygous lipoprotein lipase deficiency is estimated to be present in less than 1 in 500 people, cases have been detected and studied more frequently after initial observations that they can manifest as familial combined hyperlipidemia and may be at significant risk for cardiovascular disease,[42] particularly if risk factors such as abdominal obesity, hyperinsulinemia, estrogen treatment, or excess alcohol intake are present.[38] This was evident in the maternal aunt who by history developed adverse risk factors followed by severe hypertriglyceridemia associated with episodes of pancreatitis and more recently has acquired type 2 diabetes. Parental lipid profiles showed mild fasting hypertriglyceridemia in the father associated with mild increases in apoC-III in the heparin-precipitated apoB-containing fraction, consistent with increased cardiovascular risk.[39] Also, his LDL and IDL particle concentrations were above the mean and greater than in the mother, although she was observed to have postprandial hypertriglyceridemia (triglyceride >2000 mg/dL) in a random sample drawn at a clinic visit.

After more than a year of observation, we conclude that the addition of orlistat to a low-fat diet is effective and safe in reducing fasting triglyceride levels and the risk for pancreatitis in siblings with severe compound heterozygous lipoprotein lipase deficiency. However, despite intensive dietary fat restriction and pancreatic lipase inhibition with orlistat, HDL-C remains low and lipoprotein abnormalities are present in chylomicron-free plasma that could lead to long-term cardiometabolic risk in the affected children and to some extent in their carrier parents. These observations support careful monitoring of cardiovascular risk, lifestyle prescription and additional treatment when indicated.

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References

1. Foubert L, De Gennes JL, Lagarde JP, et al. Assessment of French patients with LPL deficiency for French Canadian mutations. *J Med Genet*. 1997;34:672–675.
2. Gotoda T, Shirai K, Ohta T, et al. Diagnosis and management of type I and type V hyperlipoproteinemia. *J Atheroscler Thromb*. 2012;19:1–12.
3. Brunzell JD, Iverius PH, Scheibel MS, et al. Primary lipoprotein lipase deficiency. *Adv Exp Med Biol*. 1986;201:227–239.
4. Gaudet D, Methot J, Dery S, et al. Efficacy and long-term safety of alipogene tiparvovec (AAV1-LPL(S447X)) gene therapy for lipoprotein lipase deficiency: an open-label trial. *Gene Ther*. 2012 in press http://dx.doi.org/101038/gt.2012.43.
5. Carpentier AC, Frisch F, Labbe SM, et al. Effect of alipogene tiparvovec (AAV1-LPL(S447X)) on postprandial chylomicron metabolism in lipoprotein lipase-deficient patients. *J Clin Endocrinol Metab*. 2012; 97:1635–1644.
6. Gaudet D, Methot J, Kastelein J. Gene therapy for lipoprotein lipase deficiency. *Curr Opin Lipidol*. 2012;23:310–320.
7. Riepponen P, Marniemi J, Rautaoja T. Immunoturbidimetric determination of apolipoproteins A-1 and B in serum. *Scand J Clin Lab Invest*. 1987;47:739–744.
8. Alaupovic P, Tavella M, Fesmire J. Separation and identification of apo-B-containing lipoprotein particles in normolipidemic subjects

and patients with hyperlipoproteinemias. *Adv Exp Med Biol*. 1987; 210:7–14.
9. McConathy WJ, Koren E, Wieland H, et al. Evaluation of immunoaffinity chromatography for isolating human lipoproteins containing apolipoprotein B. *J Chromatogr*. 1985;342:46–66.
10. Otvos JD, Jeyarajah EJ, Bennett DW, Krauss RM. Development of a proton nuclear magnetic resonance spectroscopic method for determining plasma lipoprotein concentrations and subspecies distributions from a single, rapid measurement. *Clin Chem*. 1992;38:1632–1638.
11. Tzotzas T, Krassas GE, Bruckert E. Administration of orlistat in a patient with familial hyperchylomicronemia. *Atherosclerosis*. 2002;165: 185–186.
12. Tolentino MC, Ferenczi A, Ronen L, Poretsky L. Combination of gemfibrozil and orlistat for treatment of combined hyperlipidemia with predominant hypertriglyceridemia. *Endocr Pract*. 2002;8:208–212.
13. Wierzbicki AS, Reynolds TM, Crook MA. Usefulness of orlistat in the treatment of severe hypertriglyceridemia. *Am J Cardiol*. 2002;89: 229–231.
14. Brisson D, Methot J, Tremblay K, Tremblay M, Perron P, Gaudet D. Comparison of the efficacy of fibrates on hypertriglyceridemic phenotypes with different genetic and clinical characteristics. *Pharmacogenet Genomics*. 2010;20:742–747.
15. Rouis M, Dugi KA, Previato L, et al. Therapeutic response to medium-chain triglycerides and omega-3 fatty acids in a patient with the familial chylomicronemia syndrome. *Arterioscler Thromb Vasc Biol*. 1997; 17:1400–1406.
16. Matern D, Seydewitz H, Niederhoff H, Wiebusch H, Brandis M. Dyslipidaemia in a boy with recurrent abdominal pain, hypersalivation and decreased lipoprotein lipase activity. *Eur J Pediatr*. 1996;155:660–664.
17. Viner RM, Hsia Y, Tomsic T, Wong IC. Efficacy and safety of anti-obesity drugs in children and adolescents: systematic review and meta-analysis. *Obes Rev*. 2010;11:593–602.
18. Hsieh CJ, Wang PW, Liu RT, et al. Orlistat for obesity: benefits beyond weight loss. *Diabetes Res Clin Pract*. 2005;67:78–83.
19. Anderson JW. Orlistat for the management of overweight individuals and obesity: a review of potential for the 60-mg, over-the-counter dosage. *Expert Opin Pharmacother*. 2007;8:1733–1742.
20. Henness S, Perry CM. Orlistat: a review of its use in the management of obesity. *Drugs*. 2006;66:1625–1656.
21. McDuffie JR, Calis KA, Booth SL, Uwaifo GI, Yanovski JA. Effects of orlistat on fat-soluble vitamins in obese adolescents. *Pharmacotherapy*. 2002;22:814–822.
22. McDuffie JR, Calis KA, Uwaifo GI, et al. Three-month tolerability of orlistat in adolescents with obesity-related comorbid conditions. *Obes Res*. 2002;10:642–650.
23. Gilbert B, Rouis M, Griglio S, de Lumley L, Laplaud P. Lipoprotein lipase (LPL) deficiency: a new patient homozygote for the preponderant mutation Gly188Glu in the human LPL gene and review of reported mutations: 75 % are clustered in exons 5 and 6. *Ann Genet*. 2001;44:25–32.
24. Peterson J, Ayyobi AF, Ma Y, et al. Structural and functional consequences of missense mutations in exon 5 of the lipoprotein lipase gene. *J Lipid Res*. 2002;43:398–406.
25. Persson B, Bengtsson-Olivecrona G, Enerback S, Olivecrona T, Jornvall H. Structural features of lipoprotein lipase. Lipase family relationships, binding interactions, non-equivalence of lipase cofactors, vitellogenin similarities and functional subdivision of lipoprotein lipase. *Eur J Biochem*. 1989;179:39–45.
26. Reina M, Brunzell JD, Deeb SS. Molecular basis of familial chylomicronemia: mutations in the lipoprotein lipase and apolipoprotein C-II genes. *J Lipid Res*. 1992;33:1823–1832.
27. Zheng C, Khoo C, Furtado J, Sacks FM. Apolipoprotein C-III and the metabolic basis for hypertriglyceridemia and the dense low-density lipoprotein phenotype. *Circulation*. 2010;121:1722–1734.
28. Eisenberg S, Patsch JR, Sparrow JT, Gotto AM, Olivecrona T. Very low density lipoprotein. Removal of apolipoproteins C-II and C-III-1 during lipolysis in vitro. *J Biol Chem*. 1979;254:12603–12608.
29. Alaupovic P. David Rubinstein Memorial Lecture: the biochemical and clinical significance of the interrelationship between very low density and high density lipoproteins. *Can J Biochem*. 1981;59: 565–579.
30. Tomiyasu K, Walsh BW, Ikewaki K, Judge H, Sacks FM. Differential metabolism of human VLDL according to content of ApoE and ApoC-III. *Arterioscler Thromb Vasc Biol*. 2001;21:1494–1500.
31. Tanaka Y, Lin-Lee YC, Lin-Su MH, Chan L. Intestinal biosynthesis of apolipoproteins in the rat: apoE and apoA-I mRNA translation and regulation. *Metabolism*. 1982;31:861–865.
32. Lassel TS, Guerin M, Auboiron S, Chapman MJ, Guy-Grand B. Preferential cholesteryl ester acceptors among triglyceride-rich lipoproteins during alimentary lipemia in normolipidemic subjects. *Arterioscler Thromb Vasc Biol*. 1998;18:65–74.
33. Tall A, Sammett D, Granot E. Mechanisms of enhanced cholesteryl ester transfer from high density lipoproteins to apolipoprotein B-containing lipoproteins during alimentary lipemia. *J Clin Invest*. 1986; 77:1163–1172.
34. Lamarche B, Uffelman KD, Carpentier A, et al. Triglyceride enrichment of HDL enhances in vivo metabolic clearance of HDL apo A-I in healthy men. *J Clin Invest*. 1999;103:1191–1199.
35. Bagatell CJ, Knopp RH, Vale WW, Rivier JE, Bremner WJ. Physiologic testosterone levels in normal men suppress high-density lipoprotein cholesterol levels. *Ann Intern Med*. 1992;116:967–973.
36. Alaupovic P, Mack WJ, Knight-Gibson C, Hodis HN. The role of triglyceride-rich lipoprotein families in the progression of atherosclerotic lesions as determined by sequential coronary angiography from a controlled clinical trial. *Arterioscler Thromb Vasc Biol*. 1997;17: 715–722.
37. Steiner G. Lipoprotein lipase in fat-induced hyperlipemia. *N Engl J Med*. 1968;279:70–74.
38. Brunzell JD, Schrott HG. The interaction of familial and secondary causes of hypertriglyceridemia: Role in pancreatitis. *J Clin Lipidol*. 2012;6:409–412.
39. Sacks FM, Alaupovic P, Moye LA, et al. VLDL, apolipoproteins B, CIII, and E, and risk of recurrent coronary events in the Cholesterol and Recurrent Events (CARE) trial. *Circulation*. 2000;102: 1886–1892.
40. Mendivil CO, Rimm EB, Furtado J, Chiuve SE, Sacks FM. Low-density lipoproteins containing apolipoprotein C-III and the risk of coronary heart disease. *Circulation*. 2011;124:2065–2072.
41. Benlian P, De Gennes JL, Foubert L, Zhang H, Gagne SE, Hayden M. Premature atherosclerosis in patients with familial chylomicronemia caused by mutations in the lipoprotein lipase gene. *N Engl J Med*. 1996;335:848–854.
42. Babirak SP, Brown BG, Brunzell JD. Familial combined hyperlipidemia and abnormal lipoprotein lipase. *Arterioscler Thromb*. 1992;12: 1176–1183.