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Diagnostic Pitfalls during Therapy for Extreme Hypertriglyceridaemia

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Summary: We report the case of a 34-year-old man with extreme hypertriglyceridaemia (276.6 mmol/l) that was corrected by diet and triple-drug therapy. No primary defect could be found despite an intensive biochemical and genetic evaluation. Early in the time course of triglyceride-lowering therapy, the composition and concentration of different lipoprotein species changed markedly. His lipoprotein profile mimicked type III hyperlipidaemia, then familial hypercholesterolaemia, and finally hyperalphalipoproteinaemia. The increase in LDL cholesterol and apolipoprotein B was paralleled by a sixfold increase in lipoprotein(a). We conclude that these different forms of hypercholesterolaemia disappear solely with a continuation of the triglyceride-lowering therapy.

Introduction

Patients with marked hypertriglyceridaemia are at increased risk for developing acute pancreatitis (1). The cause of the pancreatitis has been attributed to hydrolysis of chylomicron-triglycerides in the pancreas by intravascular pancreatic lipase. The consequent release of free fatty acids may cause microthrombosis leading to pancreatic necrosis. Because of this, patients with triacylglycerol concentrations exceeding 11.3 mmol/l must be treated both with a lipid-free diet and with triglyceride-lowering drugs (2). As a side effect of triglyceride-lowering therapy, high-density lipoprotein (HDL) cholesterol is regularly increased; and low-density lipoprotein (LDL) cholesterol may also be increased, although rarely to above-normal levels.

Case Report

Presentation and history

The patient was a non-obese 34-year-old man, who had triacylglycerol and cholesterol values of 276.6 mmol/l and 70.3 mmol/l, respectively, at admission. He had lost 3 kg of body weight during the previous weeks and complained of lack of appetite. He drank 1 litre of beer per day. The patient had no history of polydipsia, diarrhea, vertigo, chest of pain or fatigue, and was not taking any medication. The physical examination showed no abnormalities except eruptive xanthomata of the right hand. His father died at 47 years of age of sudden cardiac death. His brother had a partial resection of the pancreas at the age of 30 after acute pancreatitis. Lipid values for the brother and both parents are not known. The patient's only child had normal triacylglycerol values, but her LDL cholesterol concentration (3.70 mmol/l) was above the 95th percentile for a 9-year-old girl (3). None of his female relatives had pregnancy-associated pancreatitis (4).

Laboratory analysis

Serum and plasma were isolated by low spin centrifugation, and the samples were stored at 4 °C. Analysis of lipoproteins was performed within 24 h after blood drawing as described previously (i. e., HDL were analyzed by removal of very-low-density lipoproteins (VLDL) and chylomicrons by ultracentrifugation followed by precipitation of apolipoprotein B-containing lipoproteins of the fraction $d > 1.006$ kg/l (5). Apolipoproteins were determined by nephelometry (BNA 100, Behring). To eliminate the interference of light scattering by triglyceride-rich lipoproteins, a clearing buffer containing detergent and cholesterol esterase[1]) was added to the sample before nephelometry (6). Lipoprotein(a) (Lp(a)) was measured by a radioimmunoassay (Pharmacia). No interference by chylomicrons could be demonstrated for this assay by adding chylomicrons to samples with known Lp(a) concentrations, similar to the procedures described in l. c. (7). Apolipoprotein(a) phenotyping was performed by western blot analysis (5) and revealed heterozygosity for the isoforms S2/S2.5. The following serum assays were performed after removal of the chylomicron fraction ($S_f > 400$) by ultracentrifugation and revealed results within the reference range: creatinine, urea, thyrotropin, free thyroxine, triiodothyronine, C-reactive protein, insulin concentrations, amylase[1]), lipase[1]), aspartate aminotransferase[1]), and alanine aminotransferase[1]). Erythrocyte sedimentation rate, hepatitis serology, oral glucose test loads, and urinalysis were within the reference range.

Hyperglycerolaemia, which can falsely be taken for hypertriglyceridaemia, was excluded by normal glycerol values (8). Glycerol was measured under conditions corresponding to the enzymatic triacylglycerol assay (Boehringer Mannheim) omitting lipase. The γ-glutamyltransferase[1]), which was elevated (681 U/l) at the first presentation (reference value ≤ 27 U/l), was within the reference range after 6 weeks. On three occasions, plasma lipolytic activity (20 min after the i. v. injection of 100 IU of heparin per kg body weight) was normal in vivo (more than fourfold increase in free glycerol and free fatty acids) and in vitro (measured by the release of free fatty acids from glycerol tri[1-^{14}C]oleate emulsion (9)). Sequencing of all 10 exons of the lipoprotein lipase[1]) gene from white blood cell genomic DNA showed no mutation (9), and the demonstration of two polymorphisms excluded the absence of one copy of the lipoprotein lipase gene. He was homozygous for the apolipoprotein E isoform E3 (10), and isoelectric focusing and immunofixation of VLDL also excluded apolipoprotein C-II deficiency.

[1]) Enzymes
γ-Glutamyltransferase (EC 2.3.2.2)
Alanine aminotransferase (EC 2.6.1.2)
Amylase (EC 3.2.1)
Aspartate aminotransferase (EC 2.6.1.1)
Cholesterol esterase (EC 3.1.1.13)
Lipase (EC 3.1.1.3)
Lipoprotein lipase (EC 3.1.1.34)

Treatment

The patient was advised to keep an almost lipid-free diet (11). In addition to dietary therapy, the patient was treated with the combination of gemfibrozil (900 mg/day) (12), acipimox (750 mg/day) (13), and marine oils (14) (ω-3 fatty acids: eicosapentaenoic acid (1050 mg/day) and docosahexaenoic acid (750 mg/day)).

Outcome

Triacylglycerol values decreased to levels in the reference range within 3 weeks (fig. 1a). Concomitantly free fatty acids also decreased. After normalization of triacylglycerol values, acipimox and subsequently marine oils were discontinued (fig. 1a). The cholesterol decrease in the different lipoprotein fractions, however, was noticeably different (fig. 1b). After 15 days of treatment, LDL cholesterol rose to a maximum of 18.25 mmol/l, and the LDL particles became markedly cholesterol-enriched. The ratio of cholesterol to triacylglycerols was more than twice the reference value (fig. 1c). The increases in LDL cholesterol and LDL apolipoprotein B were accompanied by a sixfold rise in Lp(a) (fig. 1d).

At the start of therapy, HDL were small and cholesterol-poor (0.26 mmol/l), whereas the apolipoprotein A-I concentration (1.23 g/l) was close to the lower reference limit (reference range 1.25–1.60 g/l). With therapy, however, HDL became cholesterol-rich and triglyceride-poor (fig. 1c). Apolipoprotein A-I concentrations increased to high values (2.52 g/l on day 82), but because of a greater increase in cholesterol, the ratio of apolipoprotein A-I to HDL cholesterol was below normal. The ratio of free cholesterol to total cholesterol did not change during therapy.

Eight months after the start of therapy, a trial period without any drug therapy led again to a marked triglyceridaemia (26.69 mmol/l). When triple-drug therapy was restarted, the pattern of lipoprotein decrease and increase was similar to that of the first episode, but triacylglycerol and LDL cholesterol concentrations normalized within a shorter period of time. LDL cholesterol increased from 0.41 mmol/l to 9.05 mmol/l within 2 weeks and returned to 3.28 mmol/l 4 weeks after restart of therapy. During the next 6 months, while on combination therapy with gemfibrozil and marine oils, his lipid values remained normal except for elevated HDL (HDL-cholesterol 2.07–2.87 mmol/l) and elevated free fatty acids (1.73–3.33 mmol/l) (reference range 0.19–1.20 mmol/l).

Discussion

The cause for the dyslipidaemia in our patient remains unknown. He did not have a primary metabolic defect, but this finding is characteristic for patients with type V hyperlipidaemia (15). In particular, no defects in lipoprotein lipase or its essential cofactor (apolipoprotein C-II) were detected, and lipodystrophia, glycogen storage disease, *Wilson*'s disease, endogenous hypercorticoism, and autoimmune disease were excluded by examination and laboratory tests. The most probable reason for this hypertriglyceridaemia must have been his moderate alcohol consumption (about 50 g/day) in combination with other, unidentified factors. High intake of alcohol can elevate triacylglycerol concentrations by excessive stimulation of VLDL-triglyceride synthesis by the liver (16). However, mutations in the promoter region of the lipoprotein lipase gene (17) have not been excluded in this patient. Since this patient repeatedly showed normal postheparin lipolytic activity, major defects in the lipase gene region seem to be rather unlikely. Furthermore, mutations in the promoter region have been reported only in patients with familial combined hyperlipidaemia and not in patients with type V hyperlipidaemia.

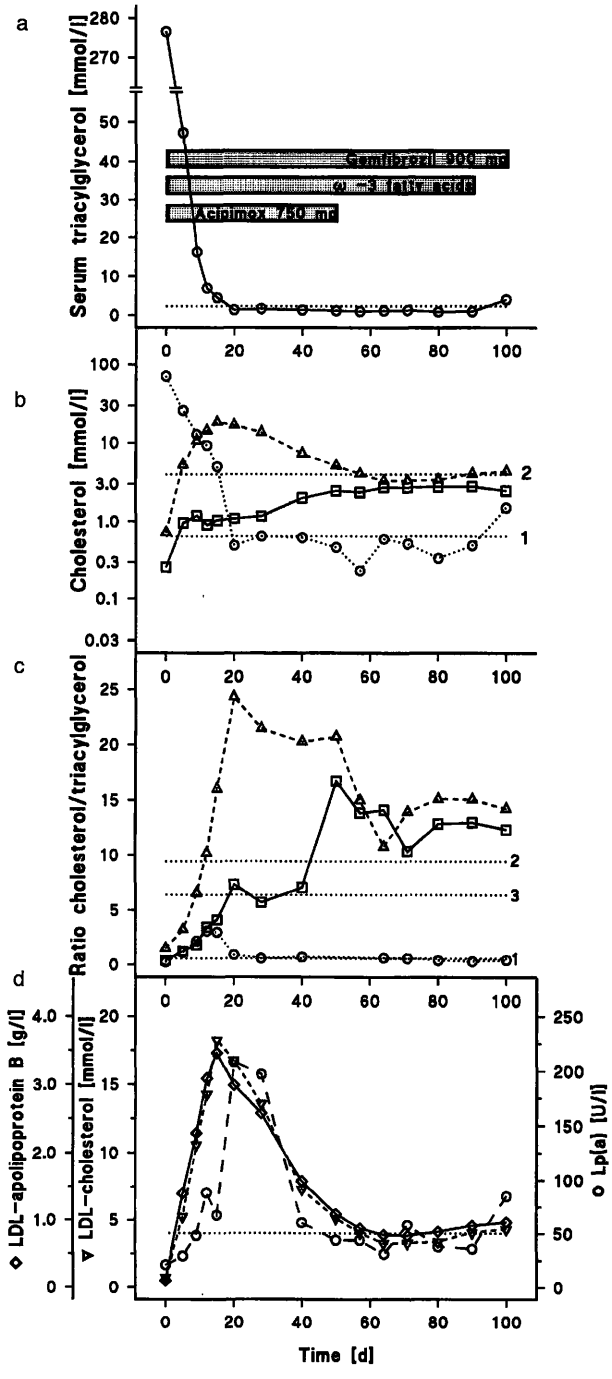

Fig. 1 Time course of serum lipids and apolipoproteins during treatment of a patient with extreme hypertriglyceridaemia.
a) Serum triacyglycerol. Dotted line indicates upper reference value.
b) Cholesterol in VLDL (○), LDL (△), and HDL (□) on a semilogarithmic scale. Dotted lines indicate upper reference value for VLDL cholesterol (1) and LDL cholesterol (2).
c) Molar ratio of cholesterol to triacylglycerol in VLDL (○), LDL (△), and HDL (□). Dotted lines indicate mean reference values [(1) VLDL, (2) LDL, (3) HDL].
d) Time course of LDL cholesterol (▽), apolipoprotein B (◇), and Lp(a) (○). Dotted line indicates upper reference value for LDL cholesterol.

The time course of triacylglycerol and cholesterol concentrations differed strikingly in this case. These data illustrate that the half-life of lipoprotein triacylglycerol in plasma is much shorter than the half-life of lipoprotein cholesterol, and thus a transient accumulation of lipoprotein remnants in different lipoprotein densities may occur (18, 19). In this patient, the accumulation of cholesterol-rich remnants first mimicked type III hyperlipidaemia (an elevation of chylomicron remnants in homozygous carriers of apolipoprotein E2), then mimicked familial hypercholesterolaemia (an elevation of LDL), and finally mimicked hyperalphalipoproteinaemia. The marked elevation of LDL cholesterol was accompanied by an increase of Lp(a), which may be due to a competition for elimination with apolipoprotein E- and apolipoprotein B-containing lipoproteins (chylomicron remnants and VLDL remnants). The composition of all lipoprotein species was noticeably altered during treatment, and similar effects would be expected to appear in many hypertriglyceridaemic patients after initiation of therapy.

Conclusion

During successful treatment of extreme hypertriglyceridaemia, marked changes in all lipoprotein classes were observed. The different half-lives of cholesterol-rich and triglyceride-rich lipoproteins can give rise to therapeutic confusion early in the treatment of extreme hypertriglyceridaemia. The accumulation of chylomicron remnants and VLDL remnants (cholesterol-rich lipoproteins of VLDL density) (20) can be taken for type III hyperlipidaemia, and a rare apolipoprotein E variant will be suspected. The accumulation of LDL-like particles might suggest a diagnosis of familial hypercholesterolaemia. However, with the continuation of triglyceride-lowering therapy without any other additional therapy, hypercholesterolaemia will disappear. High levels of Lp(a) can be observed during triglyceride-lowering therapy, and therefore Lp(a) concentration should be measured under steady-state conditions to assess the cardiovascular risk.

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