Function of hepatic triglyceride lipase in lipoprotein metabolism

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Abstract Rat hepatic triglyceride lipase (H-TGL) was purified from liver tissue extracts by affinity chromatography on Sepharose 4B with covalently linked heparin. The purified rat H-TGL exhibited the properties previously described for this enzyme. Enzyme protein was injected into rabbits for anti-H-TGL antibody production. Antirat-H-TGL did not react against rat lipoprotein lipase (LPL) but inhibited H-TGL-activity both in vitro and in vivo >90%. These antibodies were injected into rats and lipoprotein analyses were performed over a 36-hr period. It could be shown that inactivation of H-TGL by anti-H-TGL γ-globulins in vivo led to an increase in total triglyceride concentration from 70 mg/dl to 230 mg/dl due to an increase in very low density lipoprotein (VLDL) and low density lipoprotein (LDL) triglycerides 4 hr after antibody injection; a marked increase in high density lipoprotein (HDL) phospholipid concentration was observed with almost no change in HDL-cholesterol and HDL-triglycerides. This study documents the ability of antirat-H-TGL γ-globulins to inhibit H-TGL in vitro and in vivo. Furthermore, the inhibition of triglyceride removal in vivo demonstrated that this enzyme together with LPL is responsible for the catabolism of VLDL-triglyceride.—**Grosser, J., O. Schrecker, and H. Greten.** Function of hepatic triglyceride lipase in lipoprotein metabolism. *J. Lipid Res.* 1981. **22:** 437–442.

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Chylomicron and very low density lipoprotein (VLDL) metabolism occurs through multiple interactions with various enzymes. The three plasma enzymes, lipoprotein lipase (LPL), hepatic triglyceride lipase (H-TGL), and lecithin-cholesterol-acyltransferase catalyze a cascade of reactions including the hydrolysis of phospholipids at the surface of VLDL, the breakdown of triglycerides in the core, and the formation of cholesteryl esters from free cholesterol to form spherical high density lipoproteins (HDL) (1–3). The exact sequence of these events is not known, and, especially, the exact role of LPL and H-TGL during intravascular delipidation of VLDL and chylomicrons remains to be established. LPL and H-TGL differ in their molecular properties, among which the requirement of apoprotein C-II as cofactor for full activity of LPL is of particular biological interest (4–6). Studies in patients with genetically determined hyperlipoproteinemia and secondary lipid disorders in whom a selective enzyme deficiency of either LPL or H-TGL could be found indicate the different functions of both lipases in lipoprotein metabolism (7).

The purpose of this study was to verify if specific inhibition of hepatic triglyceride lipase activity in the plasma compartment with anti-hepatic triglyceride lipase γ-globulins would indeed affect the interconversion of circulating plasma lipoproteins. Analyses of the lipoprotein composition following inhibition of H-TGL would then allow a study of the specific role of H-TGL in vivo. It was found that administration of anti-H-TGL-γ-globulins led to changes of both the triglyceride as well as the phospholipid content of different lipoprotein classes.

MATERIALS AND METHODS

Purification of rat hepatic triglyceride lipase (H-TGL) and production of anti-H-TGL-γ-globulins

Hepatic triglyceride lipase was purified from rat liver tissue by a method similar to the one previously described for the purification of canine-H-TGL (8). Delipidation of rat liver tissue was performed with acetone/ether followed by extraction with 0.2 M NaCl in 0.005 M Na-barbital buffer, pH 7.4, at 4°C. The major purification step consisted of affinity chromatography on Sepharose 4B containing covalently linked heparin (Wilson Laboratories, Chicago, IL). The partially

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Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; LPL, lipoprotein lipase; H-TGL, hepatic triglyceride lipase; BSA, bovine serum albumin.
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purified enzyme was analyzed by polyacrylamide gel electrophoresis. For SDS-gels the method of Weber and Osborne was used in a slightly modified procedure (9); 7.5 and 10% gels without stacking gels were run in 0.4 M Tris-bicine buffer, pH 8.5. Gels were stained with Coomassie blue.

The purified enzyme solutions were dialyzed against 0.005 M NH_4HCO_3 at 4°C for 24 hr with three changes of the dialyzing solution. Enzyme solution was then concentrated by lyophilization, solubilized in 0.9% NaCl, and mixed with equal amounts of Freund's adjuvant. One-year-old rabbits were immunized with 100–200 μg of enzyme protein by subcutaneous injection of the antigen into the scapular regions of the animals over a period of 8 weeks. Antibody titers were assayed by immunodiffusion according to Ouchterlony (10). Rabbits were bled by heart puncture, blood was allowed to clot, and the γ-globulin fraction was isolated by Na_2SO_4 precipitation (11). Gamma-globulins were further purified by immunoadsorption on Sepharose 4B with covalently linked purified rat-H-TGL. Following elution with 3 M NaSCN, the antibody solution was dialyzed against 0.01 M phosphate buffer, pH 8.0. Antibodies were stored at −20°C. For the present studies, γ-globulins from one single rabbit were utilized.

Isolation of LPL from rat postheparin plasma and from rat epididymal adipose tissue

Rats were injected with 1000 I.U. heparin/animal into the femoral vein. Two minutes after injection they were bled by heart puncture and the plasma was separated from the blood cells by centrifugation. From the postheparin plasma the LPL was separated from H-TGL by affinity chromatography on heparin-Sepharose as described for human LPL (5). Epididymal adipose tissue was prepared and delipidated with cold acetone/ether. The delipidated powder was then homogenized with sodium barbital buffer (0.005 M, pH 7.4) and centrifuged at 800 g for 30 min at 4°C. From the clear supernatant, LPL was purified by heparin-Sepharose chromatography as previously described (12).

Animal experiments

Male Sprague Dawley rats weighing 300–350 g were used in all experiments. One polyethylene catheter was inserted into the femoral artery and another into the femoral vein of the rats. Both catheters were placed subcutaneously along the back of the animals and fixed between the ears so that the rodents could not bite the thin plastic catheters during the 36-hr experiment. Before the experiments were started, the rats had been on a commercial laboratory diet, but were fasted 18 hr prior to and during the experiments. Anti-H-TGL or control rabbit γ-globulins were injected into conscious rats according to the following schedule. Blood (0.5 ml) was obtained via the arterial catheter prior to antibody injection. At time zero each rat received a total dose of 1.5 mg anti-H-TGL over a 10-min injection period through the femoral vein catheter. Blood samples (0.5 ml) were withdrawn at 1, 2, 4, 4.5, 5, 6, 8, 23, 25, and 36 hr via the femoral artery catheter for lipoprotein and enzyme analysis. Blood volume was maintained by injecting an equal volume of physiological saline. This experimental design was carried out in nine different animals and four rats served as controls.

Triglyceride lipase assays

H-TGL and LPL were selectively measured from rat plasma following heparin injection (1000 I.U./rat). Both assay systems contained, in a total volume of 220 μl, 13.6 μmol of tri[1-^{14}C]oleoylglycerol (Amersham Buchler, Braunschweig, Germany), 55 mCi/mmol; 8.4 mmol of unlabeled triolein (Nu-Chek-Prep Inc., Elysian, MN); and 2 mg of gum arabic. The assay optimized for H-TGL contained 0.75 M NaCl, 0.2 M Tris, pH 8.8, and 10 mg of defatted bovine serum albumin (BSA) (Behring Werke, Marburg, Germany). The assay optimized for LPL contained 0.15 M NaCl and 0.2 M Tris, pH 8.2.

Selective measurement of H-TGL and LPL in rat postheparin plasma was carried out as follows. Postheparin plasma (7.5 μl) was added to 25 μl of anti-H-TGL γ-globulin solution and to 25 μl of 0.01 M phosphate buffer, pH 8.0, respectively, and allowed to stand at 4°C for 2 hr. LPL values were obtained by assaying 32.5 μl of the enzyme anti-H-TGL suspension under LPL optimized conditions. H-TGL values were obtained by assaying 32.5 μl of the enzyme-phosphate buffer suspension under H-TGL optimized conditions.

The substrate solution was prepared by sonification, with a Branson sonifier-cell disruptor (Branson Instruments Co., Stamford, CT), of a solution of gum arabic, BSA (for H-TGL-assay only), triolein, and Tris buffer in a total volume of 187.5 μl/assay or 110 μl/assay for three 1-min time periods with a setting of 3 (80 W) with cooling in ice. Care was taken not to foam the substrate solution. The assays were carried out in duplicate at 27°C. After 60 min, the reaction was terminated by addition of 1.6 ml of chloroform–heptane–methanol 75:60:85 (v/v/v) and 0.5 ml of 0.5 N NaOH to the enzyme-substrate mixture according to the method of Belfrage and Vaughan (13). After centrifugation, 0.8 ml of the upper phase was transferred into counting vials. Radioactivity was determined in a Packard Tri-Carb liquid scintillation

counter. Enzyme activity was calculated as μmol free fatty acids/ml per hr.

For the experiments shown in Fig. 1, assay conditions were slightly modified as described in the legend to this figure.

Lipoprotein analysis

VLDL, LDL, and HDL were fractionated with the Beckman airfuge, applying a method similar to the one previously described (14). One hundred fifty μl of plasma and 25 μl of a KBr solution, d 1.006 g/ml, were centrifuged for 3 hr at 100,000 rpm. Polyallomer tubes were cut and VLDL in the top was thus separated from LDL and HDL in the bottom. The infranatant was adjusted to d 1.063 g/ml and again centrifuged for 3 hr at 100,000 rpm. Separation of LDL and HDL was then achieved by slicing the tubes with the Beckman airfuge tube cutter. Cholesterol and triglycerides were determined as previously described (15, 16). Phospholipids were measured according to the method of Zilversmit and Davis (17). Protein was estimated by the method of Lowry et al. (18) with BSA as standard (18). Lipoprotein electrophoresis on agarose gels was performed as previously described (19).

RESULTS

The purified triglyceride lipase from rat liver tissue exhibited the properties previously described for this enzyme. It did not require apoprotein C-II as cofactor: it was maximally active at 0.7–1.0 M NaCl and it was not inhibited by protamine sulfate (5, 20). The enzyme preparation used for antibody production in rabbits exhibited one major band on SDS gel electrophoresis.

Fig. 1 summarizes the in vitro experiments to document the influence of anti-H-TGL γ-globulins on rat plasma H-TGL and LPL. With 80 μg of protein, more than 98% inhibition of H-TGL activity was achieved. The apparent 30% inhibition of LPL was due to the inhibition of the H-TGL activity usually observed under assay conditions optimal for LPL. Control experiments with normal γ-globulins had no effect on either H-TGL or LPL activity. Similar results were obtained with H-TGL from rat liver tissue. As shown in **Fig. 2,** anti-H-TGL γ-globulins caused no inhibition of purified LPL from rat postheparin plasma or rat epididymal adipose tissue. Results for in vivo inhibition of rat postheparin plasma H-TGL are presented in **Fig. 3.** At time zero, 1000 U of heparin was injected into a rat via the femoral vein catheter. Enzyme activity was immediately released into the blood stream and
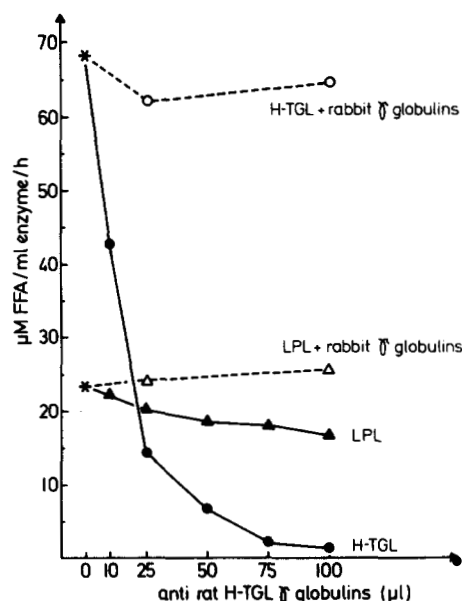

Fig. 1. Inhibition of H-TGL by antirat-H-TGL γ-globulins in vitro. 7.5 μl of rat postheparin plasma was incubated with increasing amounts of anti-H-TGL rabbit γ-globulins or control rabbit γ-globulins (0.8 mg/ml). Buffer was added to a final volume of 110 μl. After 2 hr incubation at 4°C, 110 μl of substrate was added. The samples were assayed under conditions optimal for H-TGL and LPL, respectively.

was measured over 5 hr. Five hundred μl (400 μg protein) of antirat H-TGL solution was then administered 30 min after the heparin injection when H-TGL-activity had reached its peak. Enzyme activity first totally decreased, but 30 min after antibody injection about 20% of total H-TGL-activity remained in plasma with almost no change for 5 hr. Control rabbit γ-globulins, which were injected into control animals, had no effect on H-TGL-activity.
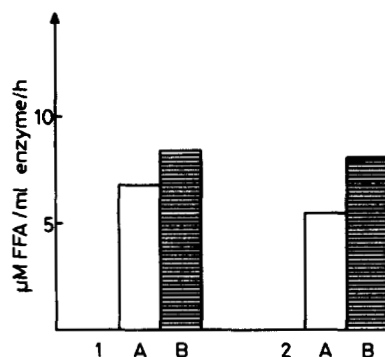

Fig. 2. Effect of anitrat-H-TGL γ-globulins on LPL activity. LPL from rat postheparin plasma, 1) and from rat epididymal adipose tissue, 2) was purified as described under Materials and Methods. Aliquots were assayed after incubation without (A) and in the presence of 80 μl (64 μg) of antirat-H-TGL γ-globulins (B) as described in the legend to Fig. 1. For full activation of LPL, 10 μl of preheparin plasma was added.
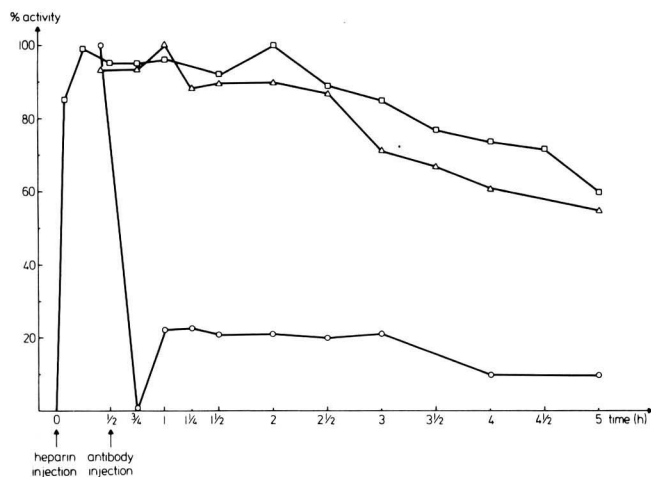
Fig. 3. Inhibition of H-TGL by antirat-H-TGL γ-globulins in vivo. H-TGL activity was assayed in the plasma of three different rats for 5 hr after injection of 1000 U heparin/animal. Rat 1 received only heparin (□ —— □), rat 2 received control rabbit-γ-globulins 30 min after heparin (△ —— △), and rat 3 received antirat-H-TGL-γ-globulins which were injected 30 min after heparin administration (○ —— ○). H-TGL-activity is expressed as percent of maximum activity after heparin injection.

Qualitative changes in lipoprotein concentration following inhibition of hepatic lipase in vivo can be visualized by looking at the lipoprotein pattern on agarose gel electrophoresis (**Fig. 4**). The pre-β-lipoproteins increased significantly with a peak between 4–8 hr. After 23 hr a normal lipoprotein spectrum was again observed. **Fig. 5a–5d** summarizes one representative experiment; similar results were obtained with eight other rats. After an initial drop, total plasma triglycerides rose dramatically from 70 to 230 mg/dl with a peak between 4 and 6 hr after antibody injection. A constant fall of the triglyceride concentration was observed, but even after 36 hr plasma triglycerides still remained at a level of 150 mg/dl. Phospholipid concentration increased rapidly from 75 to 140 mg/dl between 4 to 6 hr followed by a slow rise to a final level of 180 mg/dl. Total cholesterol gradually increased from 30 mg/dl at 2 hours to 85 mg/dl at 36 hr after injection. There was almost no change in the cholesterol and phospholipid concentration of VLDL but a striking increase in VLDL triglycerides in parallel with whole serum (Fig. 5b). A slight increase in LDL triglyceride could be observed again approximately 4–5 hr after antibody administration. LDL-cholesterol and LDL-phospholipids increased between 5 and 8 hr from 10 to 25 mg/dl and 5 to 20 mg/dl, respectively (Fig. 5c). H-TGL-blockage led to interesting effects on HDL composition. While there was almost no change in HDL-triglycerides and HDL-cholesterol, a significant increase of the phospholipid concentration from 25 to 50 mg/dl could be demonstrated (Fig.

5d). The four rats treated with control rabbit-γ-globulins showed no significant alteration in their lipid concentrations during the 36-hr period.

DISCUSSION

Plasma lipoprotein lipase and hepatic triglyceride lipase have certain common characteristics, but they clearly differ in many aspects. LPL is probably responsible for the initial hydrolysis of triglyceride-rich lipoproteins in plasma. At least in chicken, in vivo blockage of LPL by antibodies led to an immediate increase of VLDL-triglycerides as previously shown by Kompiang, Bensadoun, and Yang (21). The exact function of H-TGL in lipoprotein metabolism has not been elucidated yet. The present study describes effects caused by in vivo inhibition of H-TGL with specific antibodies in rats. The enzyme purified from liver tissue exhibited properties which were previously shown to be characteristic for hepatic lipase. The antibodies did not affect lipoprotein lipase activity, thus changes seen in the plasma lipoprotein composition following anti-H-TGL administration are due to complete or partial blockage of hepatic lipase activity alone. The increase in total triglyceride concentration from 45 mg/dl to 225 mg/dl was almost entirely due to an increase in VLDL triglycerides. However, a small yet significant increase in LDL-triglycerides could also be demonstrated in all experiments. One might speculate that this represents some form of an intermediate breakdown product of VLDL, again caused by inhibition of H-TGL in vivo.

In these experiments, rat LDL were isolated between d 1.006 and 1.063 g/ml. It is well known that in rats this particular density range may contain intermediate density lipoproteins between d 1.006 and
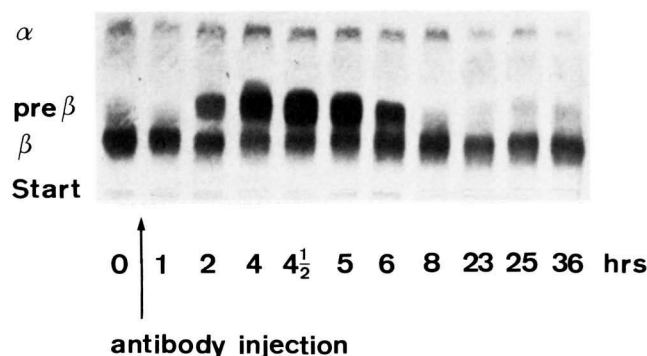

Fig. 4. Lipoprotein pattern of rat serum after antirat H-TGL γ-globulin injection. At the times indicated, blood was withdrawn via the arterial catheter of one animal and 12.5 μl volumes of serum were subjected to agarose gel electrophoresis. Lipoproteins were stained with oil red 0.
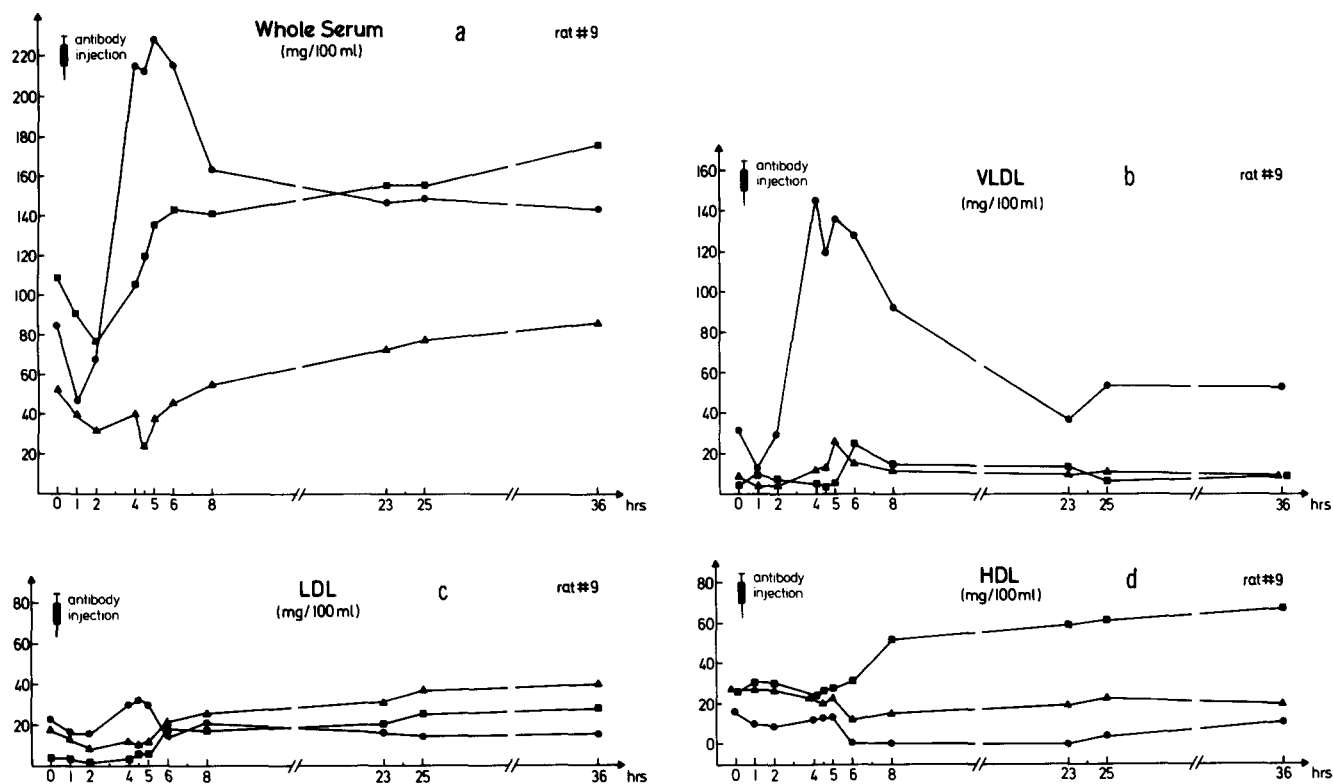
Fig. 5. Changes of lipid concentrations after in vivo H-TGL-blockage. Antirat H-TGL γ-globulins (1.5 mg) were injected into one rat via a femoral vein catheter. Blood (0.5 ml) was drawn at different time intervals for 36 hr. Total triglycerides, cholesterol, and phospholipids were determined from whole serum and isolated lipoproteins. Triglycerides, ● —— ● —— ●; cholesterol, ▲ —— ▲ —— ▲; phospholipids, ■ —— ■ —— ■.

1.019 g/ml, as well as some high density lipoproteins between d 1.05 and 1.06 g/ml in addition to the rather low concentration of low density lipoproteins. Thus, changes in the triglyceride as well as the phospholipid concentration which were observed during the course of the 36-hr experiment may stem from those lipoproteins rather than from altered LDL-particles.

The second interesting piece of information was the increase in the phospholipid concentration of HDL. It has been shown that most of the postheparin plasma phospholipase activity co-chromatographs with purified plasma hepatic triglyceride lipase and cannot be separated from H-TGL (22). One would therefore assume that the increase in HDL-phospholipid concentration is due to the block of phospholipase activity associated with hepatic lipase. A similar observation has recently been made by other investigators who also discussed this possibility as the most likely explanation for the altered phospholipid concentration in HDL (23, 24). While an increase in total triglycerides after anti-H-TGL-administration was also demonstrated in one study (23), Kuusi, Kinnunen, and Nikkilä (24) did not observe this change in VLDL concentration. The reason for this is not clear. The blocking of triglyceride removal, which

did not occur instantaneously, may lead to various speculations with regard to the site of action of hepatic lipase either in tissue or in the plasma compartment. With an intact lipoprotein lipase enzyme in these in vivo experiments, probably triglyceride hydrolysis initially starts at normal rates. The inhibition of hepatic lipase activity by specific antibodies may then lead to an increase in plasma triglycerides later in the VLDL-IDL-LDL pathway, probably not in plasma but in the liver.

One should also consider another possibility which may be of particular biological impact. If H-TGL would preferentially react with HDL₂-particles, then any inhibition of this enzyme in vivo would lead to the accumulation of such lipoproteins. These lipoproteins in turn might somehow regulate intrahepatic VLDL synthesis. Thus the observed increase in VLDL-triglycerides could be either the result of disturbed VLDL catabolism or of HDL-VLDL interaction. Further analyses of the particle size of triglyceride-rich lipoproteins, and especially of the apoprotein composition of the particles, are required and are presently under investigation in our laboratory.▉▉

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