JCI The Journal of Clinical Investigation

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J Clin Invest. 1982;70(6):1184-1192. https://doi.org/10.1172/JCI110717.

Research Article

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ABSTRACT The role of the enzyme hepatic triglyceride lipase was investigated in a primate model, the cynomolgus monkey. Antisera produced against human postheparin hepatic lipase fully inhibited cynomolgus monkey posttheparin plasma hepatic triglyceride lipase activity. Lipoprotein lipase activity was not inhibited by this antisera. Hepatic triglyceride lipase activity in liver biopsies was decreased by 65–90% after intravenous infusion of this antisera into the cynomolgus monkey. After a 3-h infusion of the antisera, analytic ultracentrifugation revealed an increase in mass of very low density lipoproteins (S_f 20–400). Very low density lipoprotein triglyceride isolated by isopycnic ultracentrifugation increased by 60–300%. Analytic ultracentrifugation revealed an increase in mass of lipoproteins with flotation greater than S_f 9 (n = 4). The total mass of intermediate density lipoproteins (S_f 12–20) approximately doubled during the 3 h of in vivo enzyme inhibition. While more rapidly floating low density lipoproteins (S_f 9–12) increased, the total mass of low density lipoproteins decreased after infusion of the antibodies. The changes in high density lipoproteins did not differ from those in control experiments.

In order to determine whether the increases of plasma concentrations of very low density lipoproteins were due to an increase in the rate of synthesis or a decrease in the rate of clearance of these particles, the metabolism of radiolabeled homologous very low density lipoproteins was studied during intravenous infusion of immunoglobulin G prepared from the antisera against hepatic triglyceride lipase (n = 3) or preimmune goat sera (n = 3). Studies performed in the same animals during saline infusion were used as controls for each immunoglobulin infusion. There was a twofold increase in the apparent half-life of the very low density lipoprotein apolipoprotein-B tracer in animals receiving the antibody, consistent with a decreased catabolism of very low density lipoproteins. Concomitantly, the rise in low density lipoprotein apoprotein-B specific activity was markedly delayed. None of these changes were observed during infusion of preimmune immunoglobulin G.

Hepatic triglyceride lipase participates with lipoprotein lipase in the hydrolysis of the lipid in very low density lipoproteins, intermediate density lipoproteins, and the larger low density lipoproteins (S_f 9–12). Thus, hepatic triglyceride lipase appears to function in a parallel role with lipoprotein lipase in the conversion of very low density and intermediate density lipoproteins to low density lipoproteins (S_f 0–9).

INTRODUCTION

Catabolism of circulating triglyceride-rich lipoproteins requires their interaction with lipolytic enzymes available to the plasma space. As a result of this process, chylomicrons are converted to smaller remnant particles before their uptake by the liver (1). Most very low density lipoproteins (VLDL)[1] are degraded to intermediate density lipoproteins (IDL) and then to low density lipoproteins (LDL) (2). This step-wise catabolism of chylomicrons and VLDL may be mediated

This work was presented in part at the 73rd Annual Meeting of the American Society for Clinical Investigation, San Francisco, CA, 27 April 1981.

Received for publication 4 February 1982 and in revised form 27 May 1982.

[1] *Abbreviations used in this paper*: Apo-B, apolipoprotein B; $F_{1.20}$, flotation rate in Svedberg units at d 1.20 g/ml; HDL, high density lipoproteins with d 1.063–1.21 g/ml, HTGL, hepatic triglyceride lipase; IDL, intermediate density lipoproteins with d 1.006–1.019 or S_f 12–20, LDL, low density lipoproteins; LPL, lipoprotein lipase; S_f, flotation rate in Svedberg units at d 1.063 g/ml; TMU-tetramethyl urea; VLDL, very low density lipoproteins with d <1.006 or S_f 20–400.

by more than one enzyme. Two lipolytic enzymes known to be present on the luminal surface of the endothelial cells are lipoprotein lipase and hepatic triglyceride lipase. The function of lipoprotein lipase (LPL) has been studied extensively over the past 25 yr and it now seems clear that this enzyme is primarily responsible for the hydrolysis of most circulating triglyceride. By contrast, the physiologic function of the hepatic triglyceride lipase is not established.

Hepatic triglyceride lipase (HTGL) is synthesized and secreted primarily by hepatocytes (3). The enzyme is available to interact with circulating lipoproteins after its attachment to the hepatic endothelial surface (4). HTGL is a major component of human postheparin lipolytic activity and, in vitro, has both triglyceride hydrolase (5) and phospholipase A-1 activities (6). Although intact chylomicrons are a poor substrate for this enzyme, HTGL has been shown in vitro to hydrolyze triglyceride in both VLDL and chylomicron remnants (7). Although no primary genetic defect of HTGL has been reported in humans to date, low levels of HTGL activity are found in chronic liver disease (8), hypothyroidism (9), and uremia (10). Abnormal triglyceride-rich lipoproteins are found in the plasma of patients with each of these conditions.

Experimental evidence for the physiologic role of HTGL has been limited to studies in the rat. Immunological inhibition of HTGL in that animal is associated with an increase in high density lipoprotein (HDL) phospholipids (11–13). This has led to the suggestion that HTGL has a role in the conversion of HDL_2 to HDL_3. In some of these studies, an increase of VLDL triglyceride was noted (12, 13). However, these experiments did not determine if this increase in VLDL was the result of a decrease in catabolism or an increase in production. In addition, these data may not be applicable to human metabolism, since in rats, most of the circulating VLDL is removed from the plasma without conversion to LDL (14). In humans, the conversion of IDL to LDL has recently been demonstrated to occur primarily across the splanchnic bed (15). Thus, an enzyme located in the liver may be involved in a pathway involving VLDL catabolism and LDL production. The cynomolgus monkey is an appropriate animal in which to study the role of HTGL, since monkeys, like humans, convert most VLDL to LDL (16). They also maintain LDL levels that are comparable to human levels. Experiments were designed to determine the changes in lipoprotein levels and composition that might occur during acute inhibition of HTGL in cynomolgus monkeys. In addition, kinetic studies of the metabolism of VLDL apolipoprotein B (apo-B) were performed to determine whether the alterations in lipoproteins were associated with a change in VLDL catabolism.

METHODS

Animals. Adult cynomolgus monkeys, females (3–4 kg) and males (4–5 kg), were maintained on a chow diet containing 5% fat (Ralston Purina, St. Louis, MO). The animals were fasted for 12–14 h before each study. Monkeys were anesthetized with intramuscular ketamine 10 mg/kg (Parke-Davis Company, Morris Plains, NJ) for liver biopsies or insertion of intravenous catheters. For studies of lipoprotein profiles or lipoprotein turnover the animals were restrained in a monkey chair and allowed to revive from anesthesia.

A total of 11 experimental animals were studied according to three different protocols involving (1) infusion of the antibody in conjunction with serial liver biopsies (n = 3), (2) serial sampling for analysis of lipoproteins by analytical and/or isopycnic ultracentrifugation (n = 5) and (3) turnover studies using radioactive VLDL (n = 3). An additional 10 animals received a preimmune goat immunoglobulin instead of the antibody. They were studied as above and served as controls. None of these 21 animals was used in more than one protocol.

In vitro studies. Postheparin plasma was obtained from normal human volunteers 15 min after intravenous injection of 60 U/kg of heparin (Riker Laboratories, Northridge, CA) and from cynomolgus monkeys 10 min after 100 U/kg of heparin. Human HTGL was partially purified and anti-human HTGL antiserum was produced in a goat as previously reported (17). In vitro assay of human postheparin plasma demonstrated comparable levels of activity after pretreatment with the antibody or with 50 mM sodium dodecyl sulfate (18) using a gum-arabic stabilized emulsion of tri-(H^3)oleoylglycerol (New England Nuclear, Boston, MA). When assayed in postheparin plasma under conditions that inhibit LPL but optimize HTGL activity (pH 8.8, 1 M NaCl) (18), addition of 1/10 vol of the antisera reduced the lipolytic activity by >90%. This inhibition did not require preincubation or centrifugation of the putative immune complex, suggesting that inactivation of HTGL occurs as a result of binding by the antibody at or near the active site.

To reduce possible nonspecific reactions of goat serum during in vivo experiments, an IgG fraction of the antiserum and of preimmune goat serum was prepared using sodium sulfate precipitation (19). The IgG was dialyzed against sterile 0.15 M NaCl containing 0.1% EDTA (pH 7.4). The goat IgG (both immune and preimmune) was treated by incubation at 4°C with Sepharose beads containing covalently bound whole monkey plasma (preheparin) to absorb nonspecific reactants. This procedure did not reduce the reactivity of the antisera against HTGL activity. Before absorption, two lines were seen on double diffusion studies with monkey sera, and these disappeared after this procedure. All IgG preparations were assayed for total protein and in vitro activity against HTGL using an artificial emulsion before their use in the monkeys.

To determine the specificity of the anti-HTGL IgG preparation, a natural substrate was used. The triglyceride in human VLDL was radiolabeled with tri-(H^3)oleoylglycerol using the method of Fielding (20). Human HTGL and LPL were partially purified by heparin-Sepharose chromatography (21). These enzyme solutions and cynomolgus monkey postheparin plasma lipase activities were assayed in a 0.5-ml assay mixture containing 0.5 mg human VLDL triglyceride (0.1 μCi/mg), 0.16 M NaCL, 12.5% bovine serum albumin, and 0.35 M tris-HCl, pH 7.4. Monkey postheparin plasma was also preincubated 30 min at 27°C, 1.0 M NaCL, pH 8.8, to completely inhibit LPL and the remaining HTGL activity was then assayed in a solution containing 1.25 M

NaCl, 8.3% bovine serum albumin, 0.233 M tris-HCl, pH 8.8. The VLDL substrate was identical to that used for the assay of human enzyme solutions. The enzyme solutions were mixed with increasing volumes of the IgG prepared from the antisera and were then added to the VLDL solution (Fig. 1). The assays were then incubated for 60 min at 27°C. Hydrolyzed free fatty acids were extracted and activity was calculated as previously described (18). All assays were performed in triplicate.

Liver biopsies. Documentation that intravenous infusion of the antibody produced inhibition of HTGL in hepatic tissue was obtained using percutaneous liver biopsy specimens. The assay was initially developed using a homogenate of cynomolgus monkey liver prepared in buffer containing 0.01 M phosphate (pH 7.4) and 0.15 M NaCl (PBS), and 0.2 U/ml heparin. Lipolytic activity was assayed in aliquots of this homogenate using 0.45 ml of gum arabic emulsion containing 5 μmol of trioleoylglycerol (10 μCi/ml) as previously described (17). Protein in the liver homogenate was measured by the method of Lowry et al. (22) using bovine serum albumin as a standard. The lipase activity was linear with increasing quantities of homogenate containing from 5 to 30 μg of protein. This activity could be completely inhibited in vitro by treatment of the homogenate with an equal volume of goat anti-human HTGL serum. No inhibition was found after incubation with preimmune goat sera.

Immediately before injection, each preparation of IgG (prepared as described above) was filtered through a 0.45-μm filter (Millipore Corp., Bedford, MA) and centrifuged 2 h at 49,000 rpm in an SW50 rotor to remove aggregates.

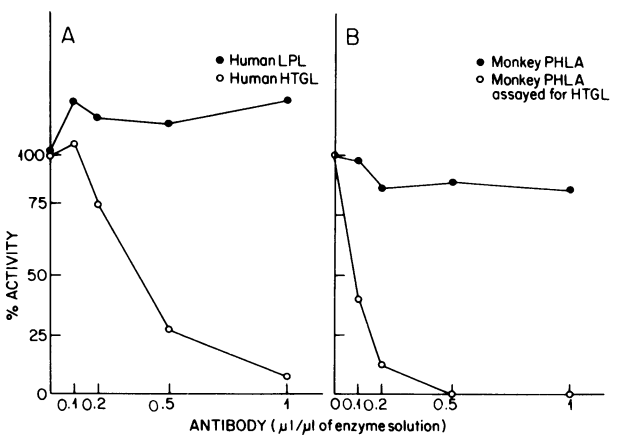

FIGURE 1 In vitro assay of anti-HTGL IgG assayed using emulsions containing tri-[H^3]oleoyglycerol in human VLDL (see Methods). A. Solutions of human HTGL (1.13 μmol of free fatty acids/ml per h) and human LPL (0.56 μmol of free fatty acids/ml per h) separated by heparin-sepharose chromatography were assayed with increasing amounts of anti-HTGL IgG (7 mg/ml). Activity at 100% in each assay was 11.2 and 56.5 nmol of free fatty acids/h for LPL and HTGL, respectively. B. Monkey postheparin plasma containing lipase activity releasing 2 μmol free fatty acids/ml per h was assayed as total lipolytic activity and after preincubation in 1.0 M NaCl, 27°C, 30 min, a condition that inactivates LPL, (0.17 μmol free fatty acid/ml per h) with increasing amounts of anti-HTGL IgG. 100% activity was 40 nmol and 5.4 nmol free fatty acid/h for total postheparin lipolytic activity and HTGL activity, respectively.

Cynomolgus monkeys were given a single rapid intravenous injection of 20 mg/kg of anti-HTGL IgG or of nonimmune goat IgG both at concentrations of 10–15 mg/ml. Percutaneous liver biopsies were obtained in duplicate (two biopsies within 2 min) using a Menghini needle before and for up to 3 h after IgG infusion. The biopsies were washed (4°C) in 1 ml of PBS, blotted on gauze, and weighed on a micro balance (Cahn Instruments, Cerritos, CA). The biopsies (5–25 mg wet wt) were placed in 150 μl of PBS containing 10 U heparin/ml and frozen (−70°C) until assayed. After defrosting, the biopsies were homogenized by passage of the material repeatedly through a 19-gauge needle using a tuberculin syringe. The effluent was then sequentially forced through a 21- and a 23-gauge needle before assay. 100-μl aliquots of homogenate were then assayed. The biopsy activity expressed per milligram of wet weight was linearly related to activity as expressed per milligram of homogenate protein. While a single intravenous bolus of anti-HTGL IgG inhibited the lipase activity, to assure maximal suppression throughout the study, an additional 20 mg/kg of anti-HTGL IgG was administered as a continuous infusion over the subsequent 3 h. This protocol was used for all subsequent experiments. Postheparin plasma LPL activity measured at the end of the infusion of anti-HTGL IgG was comparable to that obtained one day previously without IgG infusion. Rectal temperature and blood pressure were determined by hourly monitoring and were unchanged by the infusion of the IgG preparations.

Measurement of changes in lipoprotein mass. Animals were studied during infusion of anti-HTGL IgG (n = 5) and in control experiments with saline or preimmune goat IgG (n = 5). Hourly blood samples, (1.0 ml), were taken for 3–4 h before beginning the infusion to determine total plasma triglyceride and cholesterol. Enzymatic methods were used (ABA 100, Abbott Laboratories, Chicago, IL). Animals were considered to be in steady state if the plasma triglyceride and cholesterol did not change >10% during the 2 h preceding the study. Plasma samples for measurement of total plasma triglyceride and cholesterol (1-ml samples) were taken immediately before the injection of IgG. Larger samples (6–10 ml) were obtained at 5 min and at 1 and 3 h after the injection. After the initial four animals were studied, changes at 1 h were found to be similar, but less marked, than those at 3 h. The 1-h sampling was thus omitted to preserve blood volume in subsequent studies. In three control and three experimental animals, lipoprotein fractions were isolated from these larger samples by ultracentrifugation in the 40.3 rotor (Beckman Instruments, Inc., Palo Alto, CA) 39,000 rpm × 24 h, 10°C (23). VLDL (d <1.006), IDL (1.006–1.019), LDL (1.019–1.063), HDL_2 (1.063–1.12), HDL_3 (1.12–1.21) were thus obtained, and subsequently analyzed for triglyceride, cholesterol, and protein. After extraction by the method of Bligh and Dyer (24) and digestion with perchloric acid (25), total phospholipids were estimated by determination of inorganic phosphorus using malachite green (Eastman Chemicals, Rochester, NY) (26), and multiplication of this value by 25.

Analytical ultracentrifugation of the plasma from four experimental and three control animals was performed at the Donner Laboratory. The concentrations of the serum lipoproteins and their distribution as a function of flotation rate ($S_f°$ or $F_{1.20}$) were determined by computerized analysis of data from the schlieren patterns of analytical ultracentrifuge runs at 52,640 rpm of the isolated fraction containing LDL, IDL, and VLDL in a base-line solution of d 1.063 g/ml and the isolated HDL in a solution of d 1.20 g/ml (27). The flotation rates determined by a "n F° vs. p" technique

(28), are expressed in Svedbergs (10^{-13} cm S^{-1} dyn^{-1} g^{-1}) and are corrected for concentration dependence and to standard conditions (T = 26°C).

Studies of VLDL apo-B kinetics. Four to six female monkeys (not used for previous studies) were plasmapheresed, and 100–200 ml of plasma were collected into sterile tubes. VLDL was isolated by ultracentrifugation at d = 1.006 in an SW 28 rotor at 26,000 rpm, 48 h, 10°C. The VLDL was concentrated by a second ultracentrifugation in an SW 60 rotor at 55,000 rpm for 18 h. After dialysis against 0.15 M NaCl, pH 7.4, the VLDL preparation was divided and one aliquot (1 mg protein) was radioiodinated with ^{125}I and a second aliquot with ^{131}I (New England Nuclear) by the method of McFarlane (29) as modified by Bilheimer et al. (30). The radioiodinated lipoproteins were extensively dialyzed against 0.15 M NaCl, pH 7.4, 0.1% EDTA to remove free iodine and filtered (0.45 μm Millipore) before injection. The iodinated VLDL preparations were used within 3 d of labeling. The injected VLDL contained 50–75 μg of total protein and 10–20 μCi of radioactivity. Animals received a few drops of a saturated solution of potassium iodide in their drinking water for the week before each turnover study to prevent thyroidal uptake of radioactive iodine.

After intravenous injection of radioiodinated VLDL, blood samples (1.5–2.0 ml) were obtained from a separate site at 5, 15, 30 min and 1, 3, 6, and 9 h. The next day, an IgG bolus was injected as described above and 30 min later the VLDL containing the alternate iodine tracer (^{125}I or ^{131}I) was injected intravenously. Additional IgG was infused (20 mg/kg) over the subsequent 3 h. Blood was obtained as in the study the previous day. All blood samples were collected into tubes containing EDTA (3 mg/ml) and stored at 4°C after centrifugation to remove cells. A 150-μl aliquot of plasma was set aside for cholesterol and triglyceride measurements and 1 ml of plasma was used to isolate VLDL, IDL, and LDL in the 40.3 rotor as described above.

Apo-B specific activity in VLDL and IDL was determined using the method of Le et al. (31). Samples (0.9 ml) were delipidated sequentially with acetone (3 ml) and isopropyl alcohol (3 ml). The protein was solubilized with 1 ml of 9 M tetramethyl urea (TMU) (Burdick-Jackson Laboratories, Muskegon, MI) at room temperature for 1 h. Apo-B was selectively precipitated by addition of 1 ml of water and centrifugation at 300 g for 45 min. The supernatant was aspirated and the pellet washed with 3 ml of water to remove any remaining TMU. Radioactivity was determined using an autogamma scintillation spectrometer (Packard Instruments, Inc., Downers Grove, IL) and corrections made for crossover of radioactivity from ^{131}I as measured in the ^{125}I channel. Protein in each tube was determined (22) and specific activity calculated. LDL apo-B specific activity was estimated as radioactivity divided by total LDL protein.

RESULTS

Antibody activity. The specificity of the inhibition was confirmed by incubation of partially purified human HTGL with an IgG preparation of the anti-HTGL sera. This completely inhibited lipolytic activity measured using radiolabeled triglyceride in human VLDL at physiologic pH (7.4) (Fig. 1). LPL activity was not inhibited by the antibody. Cynomolgus monkey postheparin plasma measured under similar conditions was inhibited ~12% by the IgG (Fig. 1B). This decrease in activity is approximately that expected resulting from inhibition of the HTGL activity in the assayed plasma. To inhibit the LPL activity, monkey postheparin plasma was preincubated in 1.0 M NaCl for 30 min and then assayed under optimal conditions for HTGL (19). The postheparin plasma lipase activity assayed in this manner, which should have contained predominantly HTGL activity, was completely inhibited by the antibody.

The intravenous bolus of anti-HTGL IgG inhibited the hepatic triglyceride lipase activity in monkey liver biopsies by 65 to 90% (Fig. 2). The suppression was maintained for over 3 h by the infusion of the IgG. No change in lipase activity was found in similar hepatic biopsies obtained from animals receiving either preimmune goat IgG (20 mg/kg) or normal saline.

Lipoprotein masses. Analytical ultracentrifugation showed a marked rise in total VLDL mass in all animals studied after anti HTGL IgG (Fig. 3), with the greatest increase confined to the S_f 20–100 range (Fig. 4, Table I). The more rapidly floating VLDL (S_f 100–400) also increased in three of four animals. A consistent increase was also found in the S_f 12–20 lipoproteins (Table I). The S_f 0–12 fraction (LDL) fell in all animals receiving anti-HTGL IgG. Difference plots from each of these four animals (Fig. 4) showed that this fall was due to a decline in the more dense fraction (S_f 0–9). In contrast, the S_f 9–12 lipoproteins increased in all four animals after inhibition of HTGL. The three control animals showed no increase in VLDL (Fig. 3) and inconsistent changes in IDL and LDL. Analytical

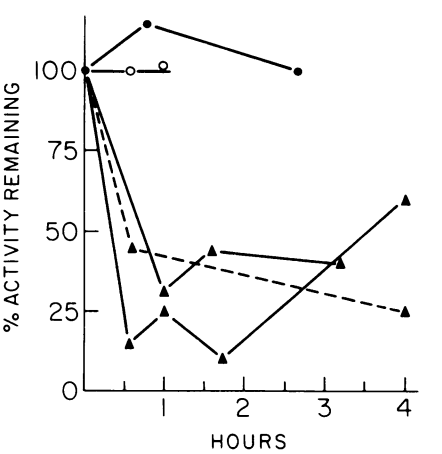

FIGURE 2 In vivo inhibition of hepatic triglyceride lipase activity. Liver biopsies were obtained at the times indicated after a single intravenous injection into the monkey of 20 mg/kg of goat anti human HTGL IgG ▲——▲, preimmune goat IgG ●——●, and saline ○——○. ▲- - -▲ indicates the activity measured after a bolus injection of IgG (20 mg/kg) followed by an equal amount of IgG infused over the ensuing 3 h. Results are of duplicate liver biopsies, homogenized and assayed for triglyceride lipase as described in Methods.

TABLE I

Lipoprotein Mass Profiles by Analytic Ultracentrifugation before and after Anti-HTGL IgG Infusion (mg/dl)

	SF 100–400		SF 20–100		SF 12–20		SF 0–12		HDL_2		HDL_3	
	0	3 h	0	3 h	0	3 h	0	3 h	0	3 h	0	3 h
Anti-HTGL IgG												
E4	0	0	3	9	3	7	92	86	4	0	131	108
E5	3	14	1	52	13	24	122	89	107	116	166	166
E6	0	12	8	35	20	34	176	141	170	184	211	179
E7	0	4	2	64	7	14	65	61	65	103	172	168
Preimmune IgG												
C3	0	0	1	1	9	15	132	148	173	228	155	168
C4	0	0	0	0	1	2	124	112	161	189	253	262
C5	7	0	25	0	5	1	105	80	94	107	187	195

ultracentrifugation demonstrated an increase in HDL_2 mass in three of four animals. However, in all three control animals, increases in HDL_2 mass of similar magnitude were observed.

The values for protein and lipids in VLDL and LDL from monkeys E-5, E-6 and E-8 are shown in Table II. VLDL triglycerides increased in all three animals with changes ranging from 60–300%. Similar increases were noted in the phospholipid content (50–380%). Animals receiving preimmune goat IgG (C-4, C-6, C-7) had no increase in VLDL lipids or protein. The IDL, as isolated by equilibrium ultracentrifugation (*d* 1.006–1.019), showed no consistent change in the mass of any component. The LDL cholesterol fell in both control and experimental animals. In the control, but not experimental monkeys, this was accompanied by a fall in LDL triglycerides. The HDL_2 phospholipid increased in all three experimental and two of three con-

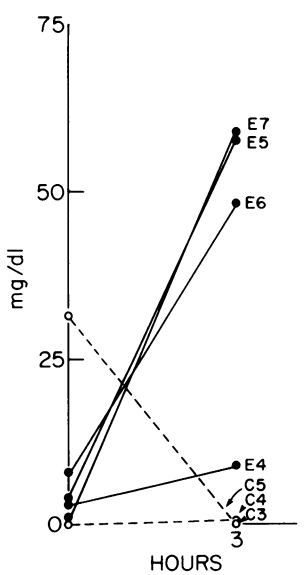

FIGURE 3 VLDL mass as determined by analytic ultracentrifugation before and after intravenous infusion of anti-HTGL IgG (E-4, E-5, E-6, E-7) and preimmune goat IgG (C-3, C-4, C-5).

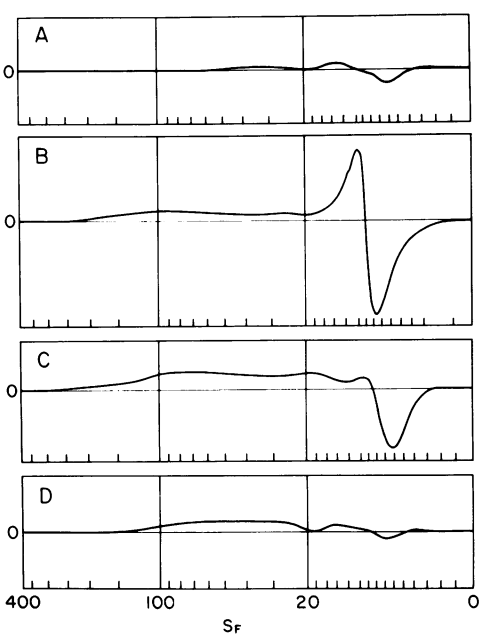

FIGURE 4 Difference plots illustrating changes in serum lipoprotein profiles after infusion of anti-HTGL IgG in four monkeys (A-E4, B-E5, C-E6, D-E7). The base-line analytical ultracentrifugal analysis is subtracted from the analysis of the sample obtained 3 h after beginning the antibody infusion. Deflections above the zero line indicate an increase in mass, whereas deflections below the line illustrate a loss of mass. The increases are of lipoproteins of S_f 9–400, whereas lipoproteins of S_f 0–9 are decreased.

TABLE II

VLDL and LDL Composition (mg/dl) before and after HTGL IgG (A) or Preimmune Goat IgG (B)

	TG		Chol*		PL*		Protein	
	0	3 h	0	3 h	0	3 h	0	3 h
A VLDL								
Animals receiving anti-HTGL IgG								
E5	10	16	1	1	1.7	2.5	4.1	4.7
E6	8	32	1	8	1.4	6.8	4.4	10.3
E8	5	8	1	1	0.6	1.2	2.8	4.6
Animals receiving preimmune goat IgG								
C4	10	11	2	2	2.0	2.6	4.1	5.3
C6	10	12	1	1	1.8	2.0	2.9	3.2
C7	25	11	2	1	3.6	1.3	4.2	2.6
B LDL								
Animals receiving anti-HTGL IgG								
E5	7	7	71	50	30	30	59	48
E6	5	7	27	22	24	20	64	69
E8	6	8	57	51	27	20	66	54
Animals receiving preimmune goat IgG								
C4	8	8	62	53	37	30	47	49
C6	10	7	38	24	27	22	30	23
C7	9	6	26	19	20	17	24	20

* TG, triglycerides; Chol, cholesterol; PL, phospholipids.

TABLE III

HDL Compositional Changes (mg/dl) Base line and after Anti-HTGL IgG (A) Or Preimmune Goat IgG (B)

	TG*		Chol*		PL*		Protein	
	0	3 h	0	3 h	0	3 h	0	3 h
A HDL_2								
Animals receiving anti-HTGL IgG								
E5	7	8	37	37	54	60	115	113
E6	6	8	15	16	36	46	81	91
E8	5	5	36	40	12	14	108	110
Animals receiving preimmune goat IgG								
C4	7	12	54	58	112	116	140	157
C6	7	8	25	28	48	57	57	59
C7	7	5	30	29	57	49	44	51
B HDL_3								
Animals receiving anti-HTGL IgG								
E5	2	5	13	10	21	20	196	159
E6	6	8	5	9	22	20	255	241
E8	2	4	11	12	39	38	102	99
Animals receiving preimmune goat IgG								
C4	2	4	26	26	43	49	156	149
C6	16	12	44	33	30	28	41	44
C7	16	15	30	35	26	31	34	46

* TG, triglycerides; Chol, cholesterol; PL, phospholipids.

trol animals when examined 3 h after IgG infusion (Table III). There were no other consistent changes in the composition of HDL_2 or HDL_3. Reflecting the VLDL increase, total plasma triglyceride values increased in each of the five monkeys receiving anti-HTGL IgG, producing a rise for this lipid of 16–140% (Table IV). A small fall in total plasma cholesterol was found in both treated and control animals.

VLDL apolipoprotein B metabolism. The effects of anti-HTGL IgG infusion on the metabolism of apo-B in VLDL, IDL and LDL were studied by intravenous injection of radiolabeled VLDL. As a representative experiment, the specific activities of apo-B in all three lipoproteins from monkeys E9 and C8 during 6 h of saline infusion are shown in the first panel of Figs. 5A and 5B, respectively. In six monkeys studied, the specific activity of apo-B in IDL and LDL rose rapidly, reaching a peak within the first hour during these baseline experiments with saline infusion (Table V). The metabolism of a second VLDL tracer, which was injected into monkey E-9 during infusion of anti-HTGL IgG, is also shown in Fig. 5A (right panel). In this and two additional experiments, (monkeys E-10 and E-11),

TABLE IV

Triglyceride and Cholesterol (mg/dl) Levels after Anti-HTGL IgG

	Triglyceride		Cholesterol	
	0	3 h	0	3 h
Anti-HTGL IgG				
E4	52	61	63	67
E5	48	57	136	115
E6	52	125	92	85
E7	35	42	144	117
E8	54	93	90	79
Control				
C3*	67	49	131	116
C4*	57	64	127	130
C6*	65	68	124	110
C7*	92	81	99	93
C8‡	47	40	89	81
C9‡	87	78	108	107

* Received intravenous preimmune IgG.
‡ Received intravenous saline.

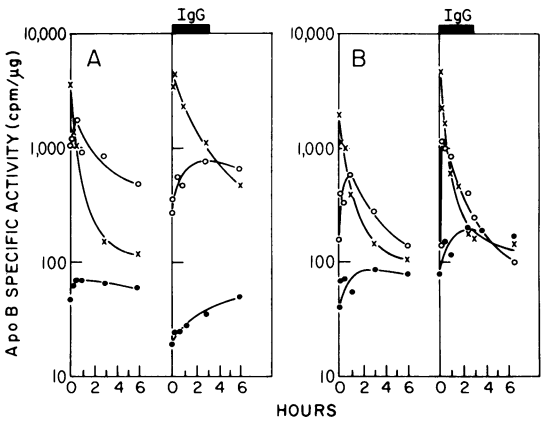

FIGURE 5 Effect of anti-HTGL IgG (A) and preimmune goat IgG (B) on VLDL apo-B metabolism. Specific activity of apo-B in VLDL XXX, IDL OOO, and LDL ●●● after intravenous injection of radiolabeled VLDL is graphed vs. time. A base-line study performed in each animal during infusion of saline is shown (A and B right panels). The same VLDL preparation labeled with a different isotope, was used for a study during an IgG infusion the following day in each animal (left panels). The anti-HTGL IgG study (A) illustrated was performed using animal E9 and the preimmune IgG study (B) illustrated is from monkey C8. The bar above the graphs indicates the period during infusion of IgG.

the VLDL apo-B specific activity fell more slowly during the inhibition of HTGL (Table V). After the antibody infusion, the increase to peak specific activity in IDL was markedly retarded reaching a maximum at ~3 h. The peak specific activity for LDL was observed between 3 and 6 h. In the three control experiments (monkeys C-8, C-9, and C-10) nonimmune IgG was infused. No evidence for slowing in the clearance of VLDL or for a delay in the conversion of VLDL apo-B to IDL and to LDL was found (Fig. 5B and Table V). The apparent half life of apo-B in VLDL increased from 0.47±0.06 to 1.0±0.43 h (x±SD) in the HTGL inhibited animals. By contrast, the half-life of VLDL apo-B tracer in control animals decreased slightly from 0.47±0.15 to 0.33±0.08 h.

DISCUSSION

In the present studies, acute inhibition of HTGL in the cynomolgus monkey produced a rise in the mass of VLDL (S_f >20), IDL (S_f 12–20) and a subclass of LDL (S_f 9–12). The smaller, more dense LDL (S_f 0–9) decreased and, as a result, the total LDL level fell. These data taken together with the specific activity curves of apo-B obtained following injection of radiolabeled VLDL, strongly support the hypothesis that in this primate, HTGL facilitates the degradation of VLDL and IDL and their subsequent conversion to LDL. Since lipoprotein lipase was not inhibited in vitro or in the postheparin plasma of animals receiving an infusion of anti-HTGL IgG, it seems safe to assume that lipoprotein lipase was active in vivo during all experiments. The continuing degradation of VLDL and the conversion to LDL observed during the turnover studies was probably due to the action of LPL. It is also possible that some residual HTGL activity was present in vivo, since intravenous infusion of the antibody produced 65–90% suppression of the baseline activity when measured in homogenates of liver biopsies. However, it is believed that this residual activity represented intracellular enzyme that was not available to the circulating IgG molecules. Such an assumption is consistent with previous estimates that 25% of HTGL activity is present within rat hepatocytes (12).

TABLE V

VLDL Apo-B Kinetics during Intravenous Infusion of Saline and Either Anti-HTGL or Preimmune IgG

	VLDL apo-B half life (h)		Peak IDL apo-B specific activity (h)		Peak LDL apo-B specific activity (h)	
	Saline	IgG	Saline	IgG	Saline	IgG
A Monkeys receiving anti-HTGL IgG						
E9	0.50	0.75	0.5	3.0	3.0	6.0
E10	0.50	1.50	1.0	3.0	1.0	6.0
E11	0.42	0.75	0.5	1.75	3.0	3.0
Mean±SD	0.47±0.05	1.0±0.43	0.67±0.28	2.58±0.72	1.42±1.24	5.0±0.173
B Monkeys receiving preimmune IgG						
C8	0.60	0.40	1.2	1.25	3.0	2.0
C9	0.50	0.25	1.0	0.5	3.0	3.0
C10	0.30	0.35	1.0	0.3	3.0	2.0
Mean±SD	0.47±0.15	0.33±0.08	1.07±0.15	0.68±0.50	3.0	2.33±0.58

After inhibition of HTGL, an increase was found in VLDL triglycerides, cholesterol, and phospholipids, giving no indication as to which lipid component of this lipoprotein might be the primary substrate for the enzyme. The decreased clearance of radiolabeled apo-B in VLDL confirmed that these mass changes were, in fact, due to inhibition of the degradation of VLDL particles and not the result of an increase in VLDL secretion. The observed doubling of the half-life of the VLDL tracer should have resulted in a twofold increase in the concentration of plasma VLDL. Thus the increases in VLDL lipids and proteins were appropriate for the degree of inhibition of VLDL catabolism.

The appearance of radiolabeled apo-B in IDL after injection of ^{125}I-VLDL was consistent with the reduced clearance of VLDL noted above. The rate of decline in the apo-B specific activity in IDL was also significantly reduced during infusion of anti-HTGL as compared to the controls, indicative of a reduction of IDL catabolism. The increase in IDL (S_f 12–20) lipoproteins as determined by analytical ultracentrifugation, indicates that the decrease in IDL catabolism was greater than the reduced input from VLDL.

The data from the analytical ultracentrifuge suggest that HTGL may be involved in the conversion of a larger and less dense LDL to the more common S_f 0–9 LDL. Such a role for HTGL is also compatible with a number of clinical observations. Women have lower mean HTGL levels (32) and larger quantities of the more rapidly floating LDL than do men (33). An increase in light LDL is inversely correlated with HTGL activity as measured in the postheparin plasma of distance runners (34).

HDL_2 phospholipids and total mass increased in both the experimental and control animals. The lack of clear changes in HDL may relate to the acute nature of these experiments. Similar experiments performed in rats have demonstrated a rise in HDL phospholipids (11–13) that was confined to the HDL_2 density range. In one of these studies (13), an increase in VLDL clearly preceded the changes in HDL. HTGL has been postulated to be the principal enzyme removing phospholipid from HDL_2, thus converting those particles to the more dense HDL_3. In view of the present findings, it is possible that the changes observed in the rat may have been secondary to altered metabolism of the lower density lipoproteins. We postulate that during degradation of triglyceride-rich lipoproteins by HTGL, surface components may be directly taken up by the liver. This process could involve apolipoprotein E, a component of VLDL, for which there is a specific receptor on the hepatocyte (35, 36). By contrast, surface components released by LPL in peripheral tissues may be transferred to HDL (37). Thus, if the two enzymes act in parallel, inhibition of HTGL would lead to increased degradation of VLDL by LPL and the transfer of a larger mass of lipids and apoproteins HDL. This hypothesis would predict a rise in HDL_2 without the need to propose a reduction in its conversion to HDL_3.

LPL may be most active with chylomicrons and larger VLDL that are rich in C apolipoproteins, whereas HTGL may be particularly important in the catabolism of particles that are deficient in the C apolipoproteins (including apolipoprotein CII, the activator of LPL). Compatible with this hypothesis, are the recent reports of reduced hepatic metabolism of VLDL and other triglyceride-rich lipoproteins after addition of a source of C apolipoproteins (38, 39). Although LPL can convert VLDL to LDL in vitro (40), this degradative process may be heavily shared with HTGL in vivo. Such a parallel role for HTGL is consistent with the continued metabolism of VLDL and the production of some LDL by human subjects with primary lipoprotein lipase deficiency (41–43).

ACKNOWLEDGMENTS

The authors would like to thank Dr. Patsy Wang-Iverson for providing the tri-[H^3]oyleoglycerol-labeled VLDL, Tibor Szabo, D.V.M. for multiple helpful suggestions, and Mrs. Marion Shiffer for assisting in preparation of this manuscript.

Supported in part by grants HL 28765, HL 23077, HL 22967, HL 18574, and Postdoctoral Research Service Award HL 06157 from the National Institutes of Health. Dr. Goldberg is a recipient of a Clinician-Scientist Award from the American Heart Association and its local affiliate, the New York Heart Association. Dr. Le is supported in part by postdoctoral traineeship HL 07343 awarded to the Division of Arteriosclerosis, College of Physicians & Surgeons, Columbia University, New York.

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