JCI The Journal of Clinical Investigation

Role of lipoprotein lipase in the regulation of high density lipoprotein apolipoprotein metabolism. Studies in normal and lipoprotein lipase-inhibited monkeys.

I J Goldberg, … , M Moukides, R Ramakrishnan

J Clin Invest. 1990;86(2):463-473. https://doi.org/10.1172/JCI114732.

Research Article

Mechanisms that might be responsible for the low levels of high density lipoprotein (HDL) associated with hypertriglyceridemia were studied in an animal model. Specific monoclonal antibodies were infused into female cynomolgus monkeys to inhibit lipoprotein lipase (LPL), the rate-limiting enzyme for triglyceride catabolism. LPL inhibition produced marked and sustained hypertriglyceridemia, with plasma triglyceride levels of 633-1240 mg/dl. HDL protein and cholesterol and plasma apolipoprotein (apo) AI levels decreased; HDL triglyceride (TG) levels increased. The fractional catabolic rate of homologous monkey HDL apolipoproteins injected into LPL-inhibited animals (n = 7) was more than double that of normal animals (0.094 +/- 0.010 vs. 0.037 +/- 0.001 pools of HDL protein removed per hour, average +/- SEM). The fractional catabolic rate of low density lipoprotein apolipoprotein did not differ between the two groups of animals. Using HDL apolipoproteins labeled with tyramine-cellobiose, the tissues responsible for this increased HDL apolipoprotein catabolism were explored. A greater proportion of HDL apolipoprotein degradation occurred in the kidneys of hypertriglyceridemic than normal animals; the proportions in liver were the same in normal and LPL-inhibited monkeys. Hypertriglyceridemia due to LPL deficiency is associated with low levels of circulating HDL cholesterol and apo AI. This is due, in part, to increased fractional catabolism of apo AI. Our studies suggest that variations in the rate of LPL-mediated lipolysis of TG-rich lipoproteins […]

Role of Lipoprotein Lipase in the Regulation of High Density Lipoprotein Apolipoprotein Metabolism

Studies in Normal and Lipoprotein Lipase–inhibited Monkeys

Ira J. Goldberg, William S. Blaner, Theresa M. Vanni, Maria Moukides, and Rajasekhar Ramakrishnan
Department of Medicine and Specialized Center of Research in Arteriosclerosis, Columbia University College of Physicians and Surgeons, New York 10032

Abstract

Mechanisms that might be responsible for the low levels of high density lipoprotein (HDL) associated with hypertriglyceridemia were studied in an animal model. Specific monoclonal antibodies were infused into female cynomolgus monkeys to inhibit lipoprotein lipase (LPL), the rate-limiting enzyme for triglyceride catabolism. LPL inhibition produced marked and sustained hypertriglyceridemia, with plasma triglyceride levels of 633–1240 mg/dl. HDL protein and cholesterol and plasma apolipoprotein (apo) AI levels decreased; HDL triglyceride (TG) levels increased. The fractional catabolic rate of homologous monkey HDL apolipoproteins injected into LPL-inhibited animals (n = 7) was more than double that of normal animals (0.094±0.010 vs. 0.037±0.001 pools of HDL protein removed per hour, average±SEM). The fractional catabolic rate of low density lipoprotein apolipoprotein did not differ between the two groups of animals. Using HDL apolipoproteins labeled with tyramine-cellobiose, the tissues responsible for this increased HDL apolipoprotein catabolism were explored. A greater proportion of HDL apolipoprotein degradation occurred in the kidneys of hypertriglyceridemic than normal animals; the proportions in liver were the same in normal and LPL-inhibited monkeys.

Hypertriglyceridemia due to LPL deficiency is associated with low levels of circulating HDL cholesterol and apo AI. This is due, in part, to increased fractional catabolism of apo AI. Our studies suggest that variations in the rate of LPL-mediated lipolysis of TG-rich lipoproteins may lead to differences in HDL apolipoprotein fractional catabolic rate. (*J. Clin. Invest.* 1990. 86:463–473.) Key words: atherosclerosis • free fatty acids • kidney • low density lipoprotein • triglyceride

Introduction

The inverse correlation between the levels of circulating high density lipoprotein (HDL) cholesterol and the incidence of coronary heart disease in Americans (1–3) emphasizes why it is important to completely understand the processes regulating the synthesis and catabolism of HDL. Production of circulating HDL requires apolipoprotein (apo) synthesis by a number of tissues; lipoprotein secretion is followed by several processes in the circulation that alter the size and composition of the HDL particle. The major protein component of HDL, apo AI, is synthesized and secreted by the liver and intestine as a component of discoidal and small spherical particles (4). These nascent HDL increase in size as they accept free cholesterol which is then converted to cholesteryl ester via the action of lecithin:cholesterol acyl transferase. Additional apolipoproteins (especially apo Cs and E) and lipid are transferred to HDL after hydrolysis of triglyceride (TG)[1] in the core of TG-rich lipoproteins (chylomicrons and very low density lipoproteins [VLDL]) by lipoprotein lipase (LPL) (4). LPL is synthesized in a variety of tissues including adipose and muscle and its interaction with circulating lipoproteins is thought to occur while the enzyme is bound to the luminal side of capillary endothelial cells (5).

HDL catabolism appears to occur via at least two pathways. The interaction of HDL with a specific lipoprotein receptor on the surface of cells, especially hepatocytes, may result in removal of some HDL from the circulation (6, 7). Alternatively, after binding to its receptor, only lipid (but not protein) may be removed from the HDL particles. Studies in rats using HDL labeled in apo AI with radioiodinated tyramine-cellobiose (TC) and also labeled with [^{3}H]cholesteryl ether (labels trapped after uptake by the cells) demonstrated that liver uptake of plasma HDL cholesteryl ether was greater than its uptake of apo AI (8). By contrast, a greater amount of apo AI than HDL lipid was removed by the kidney. Injected apo AI was found in the proximal tubule of the kidney (9). Therefore, some circulating HDL may be delipidated, perhaps via the actions of hepatic triglyceride lipase (HTGL) bound to hepatic capillary endothelium, and free apolipoprotein filtered and reabsorbed in the kidney. In some studies of human postheparin blood, the activity of HTGL was negatively correlated with levels of HDL cholesterol (10, 11) and LPL activity was positively correlated with HDL levels (11, 12). Because LPL and HTGL are located primarily on endothelial surfaces, their actions on lipoproteins resulting in these correlations probably occur in the circulation and affect HDL catabolic, rather than synthetic, events.

The studies reported here were designed to further delineate the relationship between LPL activity and circulating levels of HDL. The cynomolgus monkey has previously been used for studies of lipoprotein metabolism (13–15) and is a good model for human lipoprotein physiology. Infusion of

Address reprint requests to Dr. Goldberg, Department of Medicine, Columbia University College of Physicians and Surgeons, 630 West 168th Street, New York, NY 10032.

Received for publication 22 February 1989 and in revised form 6 April 1990.

J. Clin. Invest.

0021-9738/90/08/0463/11 $2.00
Volume 86, August 1990, 463–473

1. *Abbreviations used in this paper:* FCR, fractional catabolic rate; HTGL, hepatic triglyceride lipase; LPL, lipoprotein lipase; TC, tyramine-cellobiose; TG, triglyceride.

anti-LPL antibodies into these monkeys has been reported to rapidly (within 3 h) cause increases in plasma and HDL TG, and decreases in total HDL mass (14). We now report the effects of more prolonged LPL inhibition, and the resulting hypertriglyceridemia, on HDL apolipoprotein metabolism. In an effort to define the mechanism responsible for variations in HDL apolipoprotein fractional catabolic rate (FCR), the tissue sites of HDL protein degradation were determined and compared in normal and hypertriglyceridemic animals. These results demonstrate an important role for LPL activity in regulating rates of HDL apolipoprotein fractional catabolic rate and provide information about the mechanism responsible for the coordinate physiologic regulation of HDL and TG metabolism.

Methods

Animals. The protocols and procedures for these studies were reviewed and approved by the Columbia University Health Sciences Division Institutional Animal Care and Use Committee. Adult female cynomolgus monkeys weighing 3–4 kg were maintained on a low-fat laboratory diet (fat was ~ 5% of weight and 10% of calories; Chow, Ralston Purina, St. Louis, MO). A total of 11 monkeys were used for these studies. Three animals each underwent two kinetic studies of HDL and LDL protein, one without and one with infusion of anti-LPL antibodies. The remaining studies were done in pairs so that a control and a LPL-inhibited animal each received the identical tracers. We have previously reported the effects of infusion of nonimmune globulins on lipoprotein levels and VLDL catabolism in this species (15). The plasma cholesterol and TG levels in plasma and isolated lipoproteins were measured by enzymatic methods using an analyzer (ABA model 100 Autoanalyzer, Abbott Laboratories, Houston, TX), and HDL cholesterol was measured after precipitation of apo B–containing lipoproteins by addition of phosphotungstic acid-magnesium to plasma (16).

3 d before insertion of intravenous catheters, monkeys were placed in a jacket-swivel apparatus (Alice King Chatham Medical Arts, Los Angeles, CA) to allow them to acclimate to this condition. The animals were fasted for 12–14 h before surgery and anesthetized with intramuscular ketamine (10 mg/kg body weight) and xylazine (2 mg). Under sterile conditions the femoral vein was exposed and an intravenous catheter was inserted. The catheter was placed in a subcutaneous tunnel and exited from the back of the animal. The monkey was placed in the jacket; the catheter was threaded through a steel cable attached to the jacket and connected to a swivel on the top of the cage. This catheter was kept patent by infusion of normal saline (2–4 ml/h) and was used for infusion of antibodies and for chronic blood sampling. The animals were allowed 1 d to recover from this minor surgical procedure. They moved freely about the cage and appeared healthy and not distressed. Monkeys 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.

All blood samples were obtained from the femoral vein catheter. Before obtaining each blood sample ~ 1.5 ml of saline and 1.5 ml of blood were aspirated. Then, using a new 3-ml syringe which had been coated with a solution of EDTA (150 mg/ml) to prevent the blood from clotting, a blood sample (1.5 ml) was obtained. The initially removed blood and saline were then reinjected and the intravenous infusion restarted. Radioactive tracers were injected via an additional catheter, a 21-gauge scalp vein needle inserted into the popliteal vein of the opposite side. After injection of the tracers and 3 ml of sterile saline, the scalp vein needle was removed. The amount of blood taken from each animal was limited to 12 ml per 24 h and < 20 ml per study. This is < 10% of the animal's estimated blood volume. Owing to concerns regarding the effects of possible immunization of the monkeys with a foreign protein, no animal participated in more than one study that required injections of antibody.

Antibody preparation. Monoclonal anti-LPL IgG was prepared from ascites collected from mice which had received two intraperitoneal injections of 1 ml of 2,6,10,14-tetramethylpentadecane (pristane) (Sigma Chemical Co., St. Louis, MO) followed by an injection of 10^7 hybridoma cells that were producing antibodies against LPL (14). The IgG was isolated by precipitation with $(NH_4)_2SO_4$ followed by chromatography on diethyleaminoethyl Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ). The resulting monoclonal IgG (0.5–2 mg/ml of protein), as previously reported (14), inhibits monkey LPL activity in vitro and in vivo and does not affect HTGL activity.

Antibody infusion. For studies of LPL inhibition the animals received intravenous injections of 4–8 mg of monoclonal IgG at the initiation of the study and additional injections containing an equal mass of IgG every 10–12 h. The amounts of IgG were chosen to contain sufficient specific IgG to inhibit more than twice the amount of LPL which would have been expected in monkey plasma after intravenous heparin administration. Kinetic studies were begun ~ 12 h after LPL inhibition and the development of hypertriglyceridemia. Subsequent injections of IgG were given immediately after obtaining timed blood samples.

Preparation of lipoprotein tracers. Monkey LDL (*d* 1.020–1.063 g/ml) and HDL (*d* 1.063–1.21 g/ml) were isolated by ultracentrifugation (17), dialyzed against 0.15 M NaCl, 10 mM sodium phosphate, 0.01% EDTA, pH 7.4 (PBS), and radioiodinated with ^{125}I or ^{131}I (Amersham Corp., Arlington Heights, IL) by the method of MacFarlane (18) as modified by Bilheimer et al. (19). The lipoproteins were separated from the free iodine by gel filtration over Sephadex G-50 (Sigma Chemical Co.), then mixed with 1 ml of monkey plasma and reisolated by ultracentrifugation. This step was performed to allow some of the exchangeable apolipoproteins (Cs and E) to transfer to VLDL and for the small amount of labeled albumin to exchange with unlabeled protein. The reisolated lipoproteins were then dialyzed against PBS. ~ 5 μCi of each tracer was used for each study. HDL tracers were analyzed by polyacrylamide gel electrophoresis (PAGE) on 12.5% gels, the gels were sliced and radioactivity was determined. > 85% of the radioactivity migrated in a position corresponding to apo AI. For some studies, radiolabeled HDL was prepared using autologous labeled apo AI. Monkey apo AI was purified from delipidated HDL by gel filtration using a fast protein liquid chromatography system (FPLC, LCC 500, Pharmacia, Inc.) (20), radioiodinated using ICl, and incubated with monkey plasma (37°C for 18 h), and the labeled HDL were reisolated by ultracentrifugation.

Lipoprotein turnover studies. After intravenous injection of radioiodinated lipoproteins into the monkeys, blood samples were obtained at either 2 or 5, 15, and 30 min, and at 1, 3, and 6 h, and then approximately every 10 h for 72 h. Studies using iodinated TC-HDL were terminated at 24 h. All blood samples were collected into syringes containing EDTA and stored at 4°C after centrifugation to remove cells. Plasma radioactivity was determined by means of an autogamma scintillation spectrometer (Packard Instruments, Inc., Downers Grove, IL) and, when necessary, corrections were made for crossover of radioactivity from ^{131}I as measured in the ^{125}I channel.

Data obtained from plasma radioactivities were fitted by a sum of two exponentials using a nonlinear regression program. From the two exponentials, the FCR for HDL and LDL protein was calculated (21). Data for FCR are given as pools per hour. The numbers are the fraction of the entire mass of apoproteins in each lipoprotein (pool) removed per hour.

Lipid and lipoprotein measurements. A 100-μl aliquot of plasma from each timed sample was set aside for cholesterol and TG measurements, and 0.5 ml of plasma from all time points was used to isolated VLDL (*d* < 1.006), IDL plus LDL (*d* 1.006–1.063), and HDL (*d* 1.063–1.21) by ultracentrifugation in the 50.3 Ti rotor (Beckman Instruments, Inc., Palo Alto, CA) at 39,000 rpm, 10°C (17). The isolations were performed using 24-h centrifugations, except for HDL which was isolated after 48 h. TG, cholesterol, and protein (22) were determined in VLDL, LDL, and HDL of these isolated samples. Apo AI in monkey plasma was measured by radioimmunoassay employing

anti-human apo AI antiserum (23) and a standard curve using monkey apo AI. VLDL and HDL apoproteins were separated by PAGE using 2–16% gradient gels (Pharmacia Fine Chemicals) (24) and stained with Coomassie R-250. A laser densitometer (model 222-020 UltraCan XL, LKB Instruments, Inc., Gaithersburg, MD) was used to scan the gels at 633 nm. All gels were scanned within the highly reproducible linear range of absorbance (< 4.0 absorbance units full-scale).

Tissue uptake of HDL protein. Radioiodinated and TC-labeled monkey HDL and TC-labeled apo AI exchanged onto HDL were prepared as described by Pittman et al. (25). TC was kindly supplied by Dr. R. Pittman, University of California, San Diego. As described above, the labeled lipoproteins were mixed with monkey plasma and reisolated by ultracentrifugation before use. 2–5 μCi of labeled HDL was injected into a control and paired LPL-inhibited monkey. 24 h later the animals were killed by pentobarbital overdose. While the heart was still beating, catheters were inserted into the left ventricle and atrium and the animal was perfused with 3 liters of cold saline. Organs were obtained and the total radioactivity and percentage of trichloroacetic acid (TCA)–precipitable radioactivity in each organ were determined (25). The percentage of tracer removed by each tissue was obtained by dividing the amount recovered in each organ by the amount of tracer initially injected. By using the initial and 24-h blood sample, the percentage of injected radioactivity remaining in the plasma was estimated. For comparison purposes, data were normalized for an injection of 5 μCi.

Results

Effects of LPL inhibition on plasma lipids. Injection of anti-LPL antibodies resulted in a rapid and sustained increase in circulating TG. As shown in Fig. 1, by 20 h after the infusion of antibodies the plasma TG appeared to reach a new steady state. Table I lists the baseline TG, cholesterol, and HDL cholesterol levels for the animals studied and the maximum plasma TG level which was observed during LPL inhibition. The maximum TG level was somewhat variable from monkey to monkey and ranged from 633 to 1,240 mg/dl. It should be noted that the animals ate their usual low fat diet during the study. We anticipate that much higher levels of plasma TG, as is seen in human LPL deficiency, might have occurred with increased fat intake.

In several monkeys, plasma TG was monitored for several days after the completion of an LPL inhibition study. The passage of 2–3 d was required, after discontinuing the antibody infusion, for the plasma TG level to return to baseline. The prolonged elevation of TG observed here probably reflects the time required for the clearance of the injected antibody (26), rather than reflecting the resynthesis of LPL.

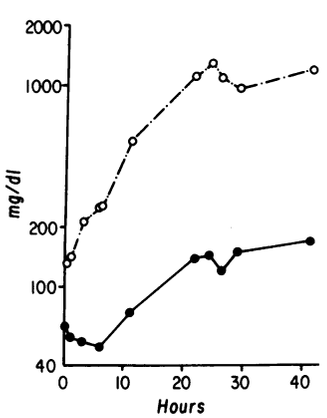

Figure 1. Changes in (●) plasma cholesterol and (○) TG after infusion of anti-LPL monoclonal antibodies. Blood samples were obtained before and during infusion of anti-LPL IgG in a Chow-fed cynomolgus monkey. The bloods were obtained via a femoral vein catheter as described in Methods. This study was performed in monkey 1.

Table I. Plasma Lipids of Monkeys

Monkey	Baseline TG	Baseline Cholesterol	Baseline HDL cholesterol	TG peak during inhibition*
		mg/dl		*mg/dl*
1	55	154	69	633
2	43	185	68	—
3	23	90	45	1,240
4	33	149	78	847
5	21	109	45	886
6	132	210	54	866
7	162	193	60	—
8	22	98	35	1,064
9	94	91	49	—
10	63	96	39	1,194
11	86	107	48	—

* Peak TG levels are not listed for animals that had only a control study performed. Animals were maintained on a Chow diet. TG and cholesterol were measured by enzymatic methods and HDL cholesterol was determined after precipitation of the apo B–containing lipoproteins with phosphotungstic acid-magnesium.

Changes in lipoprotein mass and composition during LPL inhibition. LPL inhibition resulted in marked changes in the amounts and composition of VLDL, LDL, and HDL. Changes in lipoprotein profiles assessed by analytic ultracentrifugation and changes in plasma and lipoprotein lipid composition during acute LPL inhibition have been reported previously (14). Average plasma lipid and lipoprotein levels during more prolonged LPL inhibition are shown in Table II. In Table III are shown the average lipid and lipoprotein levels for each monkey during the kinetic studies which began ~ 12 h after LPL inhibition. LPL inhibition produced a rapid and sustained increase in VLDL TG. The increase in the relative amount of TG vs. protein was consistent with the previously reported (14) increase in larger, more buoyant VLDL. There was some variability between animals in the maximum VLDL TG level achieved. Both LDL and HDL were enriched in TG content relative to cholesterol and protein (Table II). In fact, after prolonged LPL inhibition, HDL contained greater amounts of TG than cholesterol. The protein concentration of HDL and LDL decreased during the antibody infusion; for LDL the rate of this decline was most marked during the first 24 h after LPL inhibition. The decrease in HDL protein mass was reflected in a marked reduction of plasma levels of apo AI (70%) determined by radioimmunoassay. The total mass of HDL core lipid and proteins decreased during the study. This decrease in both HDL lipid and protein has previously been reported (14) and is due to a decrease in both subclasses of HDL.

VLDL and HDL apoproteins, were assessed by PAGE of isolated lipoproteins in three separate studies and a representative gel is shown in Fig. 2. During LPL inhibition, VLDL apo B and apo Cs increased and a slight increase in apo AI was noted. HDL apo AI decreased and there was a marked reduction in the band containing apo Cs and apo AII, a 9.9-kD monomeric protein in the monkey (27). The less marked visual decrease in the apo AI band compared to the immunoas-

Table II. Time Course of Lipids and Lipoprotein Changes after LPL Inhibition

	Plasma	VLDL	HDL	LDL
		mg/dl		
Baseline				
TG	103±15	22±3	15±1	12±1
Cholesterol	92±3	4±0.2	36±2	29±2
Protein	142±10*	6±1	130±4	31±3
12–22 h				
TG	499±39	359±26	21±2‡	17±2‡
Cholesterol	86±3	51±6	13±1‡	9±1‡
Protein	87±3*	58±3	100±12	18±2
23–32 h				
TG	691±26	568±30	18±3‡	21±3‡
Cholesterol	100±5	57±2	8±2‡	8±1‡
Protein	59±10*	89±6	75±3	18±3
>32 h				
TG	736±49	384±49	14±2‡	22±2‡
Cholesterol	105±4	68±11	8±1‡	10±1
Protein	41±2*	68±7	46±4	18±2

The average (±SEM) lipids and protein were calculated for each animal (n = 7) during the indicated time periods after LPL inhibition and the data from all animals were then averaged. The times indicated are after the initial injection of anti-LPL antibody. Kinetic studies were begun ~ 12 h after LPL inhibition.
* Plasma apo AI levels by radioimmunoassay (n = 5, data not available for the studies performed in monkeys 3 and 10).
‡ n = 6, data not available for monkey 5.

Table III. Lipids and Lipoproteins in Each Monkey during LPL Inhibition

Animal	Plasma	VLDL	HDL	LDL
		mg/dl		
1				
TG	490±12	490±11	15±1	18±2
Cholesterol	110±1	59±8	11±0.2	14±1
Protein	—	99±14	64±2	21±1
3				
TG	763±39	497±38	30±1	20±2
Cholesterol	104±4	150±25	13±1	10±0.4
Protein	—	103±5	74±3	20±1
4				
TG	488±24	365±48	12±1	13±1
Cholesterol	73±1	38±3	7±1	6±1
Protein	—	65±22	71±4	11±2
5				
TG	761±28	458±69	ND	ND
Cholesterol	115±3	58±6	ND	ND
Protein	—	56±3	38±2	13±1
6				
TG	660±95	358±58	16±5	28±1
Cholesterol	87±11	81±6	5±1	10±1
Protein	—	47±4	26±3	22±3
8				
TG	518±69	300±58	14±1	42±5
Cholesterol	88±4	44±6	11±2	27±5
Protein	—	29±5	69±6	38±1
10				
TG	698±37	464±22	27±1	11±1
Cholesterol	105±2	53±2	10±0.3	4±0.2
Protein	—	79±2	82±2	10±1

The average (±SEM) plasma lipids and VLDL, HDL, and LDL lipids and protein for each LPL-inhibited animal were determined on timed plasma samples obtained during the kinetic studies. The kinetic studies were begun ~ 12 h after the initial dose of anti-LPL antibody. Abbreviation: ND, not done.

say or Lowry protein determinations (Table II) may be due to the overloading of the gel required to clearly show the changes in other apolipoproteins. Densitometric scanning of this gel demonstrated that the percentage of apo Cs compared to total protein in HDL decreased from 20% to 9%; apo Cs in VLDL increased from 20% to 58% of total VLDL protein during the study.

Kinetics of LDL and HDL turnover. HDL protein turnover was assessed using three different methods of HDL labeling: exchange of apo AI onto HDL, radioiodination of whole HDL and reisolation of the labeled HDL after allowing for exchangeable apoproteins, apo Cs and E, to transfer to VLDL, and using ^{125}I-TC to label HDL. Recent data of Melchior and Castle (28) have suggested that all plasma apo AI tracers may not be associated with HDL in hyperlipidemic monkeys. Thus, the reisolation of the tracer in the range of HDL before use verified that all the injected label was initially on HDL. When the distribution of radioactivity was assessed in the initial sample obtained after injection of the tracer into the monkeys, a slight increase in the amount of tracer found in the nonlipoprotein fraction of plasma, $d > 1.21$ g/ml, was found in the LPL-inhibited monkeys. In the studies in which this was assessed (n = 5 pairs), after 24 h the percentage of the total plasma radioactivity in the nonlipoprotein fraction averaged 9.0±2.4% in the control monkeys and 14.0±3.4% in the LPL-inhibited animals (mean±SEM, P = 0.08 by paired t test). In the LPL-inhibited plasmas, an insignificant increase in tracer recovered in lipoproteins of lower density than HDL was also noted, 12.4±2.4% vs. 9.6±1.2% (P = 0.34).

As presented in Table IV, all three methods gave comparable FCRs for control HDL turnover studies. Moreover, unlike the data previously obtained by our laboratory for VLDL turnovers in cynomolgus monkeys (13, 15), the FCR for HDL protein was relatively uniform among these Chow-fed monkeys. LPL inhibition resulted in an increase in the FCR of HDL protein, or apo AI exchanged onto HDL, as compared with the same animal during a control study, or as compared with a paired animal studied at the same time. Representative studies showing plasma decay curves of HDL tracers injected into an experimental and paired control animal are shown in Fig. 3 *A* and data from seven control and seven experimental studies are given in Table IV. The HDL FCRs for control and experimental monkeys were 0.037±0.001 and 0.094±0.009 pools per hour (mean±SEM). The HDL FCR during LPL inhibition was significantly increased over that in control monkeys ($P < 0.01$, using the Wilcoxon rank sum test). Animal no. 3 attained the highest level of plasma TG during the inhibition and the greatest increase in HDL protein FCR. The tracer, ^{125}I-apo AI, used for the kinetic study in monkey 3 was

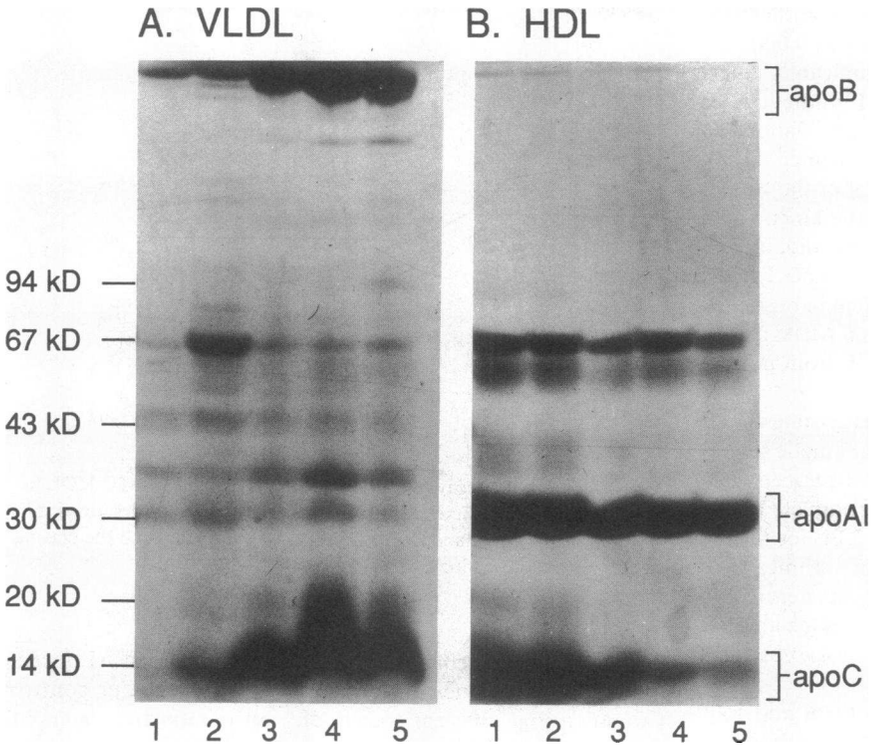

Figure 2. Changes in the levels of VLDL and HDL apoproteins after infusion of anti-LPL antibodies. VLDL and HDL were isolated from plasma samples (0.5 ml) obtained at 0 and 4 min, and 12, 24, and 32.5 h (respectively lanes *1, 2, 3, 4,* and *5*) after infusion of the anti-LPL antibody into monkey 1 (see Fig. 1 for plasma lipid levels). The recovered VLDL and HDL were lyophilized and resuspended in uptake buffer, and 5% of the recovered proteins were used for SDS-PAGE, 2–16% gradient gels. The gels were stained using Coomassie R-250 and scanned.

Table IV. Turnover of LDL and HDL in Normal and LPL-inhibited Monkeys

	HDL		LDL	
	Control	LPL inhibited	Control	LPL inhibited
	FCR in pools of protein removed per hour			
Animal				
1	—	0.063	—	0.107
2	0.030	—	0.101	—
3	0.048	0.256	0.110	0.141
4	0.031	0.062	0.167	0.092
5	0.040	0.061	0.079	0.105
6	—	0.081	—	—
7	0.038	—	—	—
8	—	0.087	—	—
9	0.034	—	—	—
10	—	0.051	—	—
11	0.039	—	—	—
Average	0.037	0.094	0.114	0.111
SEM	±0.001	±0.010	±0.009	±0.005

All studies were performed in pairs with a control and LPL-inhibited monkey receiving the same tracers. Three monkeys (3, 4, and 5) each had a control and experimental study. LDL turnover studies were performed only in animals 1–5. HDL turnover studies were performed using either radiolabeled HDL or ^{125}I-apo AI HDL (studies 2, LPL-inhibited 3, 6, and 7) prepared as described in Methods. Data from studies 8–11 was obtained using radioiodinated TC tracers. Data was calculated using plasma decay of each tracer. The increased HDL FCR during LPL inhibition was significantly different from that of control monkeys ($P < 0.01$) by the Wilcoxon rank-sum test.

also used for the control study in monkey 2 and, therefore, the rapid FCR noted during this LPL inhibition study did not appear to be due to an abnormality of the tracer. If the data from monkey 3 are eliminated, the kinetic differences between the other six control and LPL-inhibition studies were still significant ($P < 0.05$) by paired t test.

Absolute synthetic rates of HDL proteins or apo AI can be estimated by multiplying the FCRs in Table IV by the HDL protein or plasma apo AI concentrations given in Table II. By using this method, the average synthetic rate of apo AI in control studies was found to be 0.052 mg/h; the average apo AI synthetic rate in LPL-inhibited studies was 0.058 mg/h. The

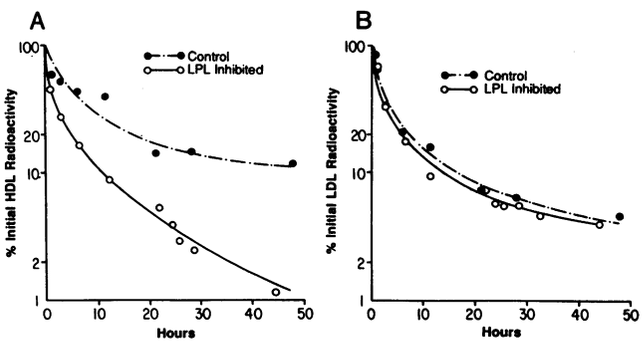

Figure 3. Catabolism of HDL and LDL in normal and LPL inhibited monkeys. (*A*) A representative study in which the turnover of radioiodinated HDL protein was studied in monkey 4 before and after LPL inhibition. (*B*) LDL protein turnover was studied in monkeys 1 and 2 after injection of the identical LDL preparation into each animal. The data shown are the plasma decay curves of each tracer.

average HDL protein synthetic rate in control studies was 0.049±0.3 mg/h and in LPL-inhibited studies was 0.060±2.2 mg/h. These latter synthetic rates were not significantly different from each other (P = 0.65). Thus, apo AI synthetic rates were not decreased by inhibition of LPL. Such calculations do require some assumptions, especially when performed during such an acute intervention as LPL inhibition. For the studies using HDL protein tracers, they assume that the kinetic data and protein estimates represent apo AI; the tracer did, in fact, contain a small amount (< 15% of the total) of other labeled protein. In addition, they assume that the protein in reisolated HDL is the same as that of the tracer, mostly apo AI. As shown in Fig. 2, most, but not all, the protein in HDL from normal and hypertriglyceridemic plasma was apo AI.

Calculations of synthetic rates assumes steady-state conditions. For this reason, rather than beginning our kinetic studies when we first injected the anti-LPL antibody, the tracers were injected 12–15 h later. Conceivably, a more complete steady state might have resulted if we had inhibited LPL for several days before beginning the kinetic studies. For technical reasons, including the amount of time which the catheters would remain patent without heparin in the infusate and the amount of purified antibody required, it was not practical to perform studies of longer duration.

Data for LDL kinetic studies in four control and four LPL-inhibited monkeys are given in Table IV and a representative study is shown in Fig. 3 *B*. No significant change in LDL protein FCR was seen. Thus, despite the marked reductions in LDL mass that occur with LPL inhibition, the FCR of that lipoprotein was unchanged. A larger number of studies may have demonstrated a change in LDL FCR. In contrast to the situation for HDL, the decreases in LDL mass were not associated with dramatic changes in catabolism, suggesting that the reduction of plasma LDL was primarily due to decreased production of this lipoprotein. In a previous report (14), we showed that LPL inhibition blocks LDL production by preventing the conversion of VLDL to LDL. The lack of effect of LPL inhibition on the fractional rate of LDL catabolism, furthermore, demonstrates that the changes in HDL FCR were specific and not due to a generalized effect associated with administration of the monoclonal antibody or associated with changes in plasma volume. In this regard, plasma volumes of the monkeys calculated from the distribution of injected tracer were not significantly different, 164±52 ml (average±SD) in control studies and 186±44 ml in LPL-inhibited studies.

Tissue uptake of HDL. To determine whether labeled intact HDL, or HDL that had labeled apo AI exchanged onto the lipoprotein, should be used for studies of tissue uptake of HDL protein, an initial experiment was performed in which ^{131}I-TC-HDL and ^{125}I-TC-apo AI HDL were simultaneously injected into a monkey. Fig. 4 shows the plasma decay curves for the two labels. The TC-apo AI was removed slightly more rapidly (FCRs of 0.026 for TC-apo AI vs. 0.038 pools per hour for TC-labeled whole HDL). Apo AI kinetic studies in humans performed using labeled apo AI have been compared to simultaneous studies in which labeled HDL was used. In one study, radioiodinated apo AI injected into humans was found to have a more rapid FCR than labeled apo AI on HDL (29); other investigators have, however, reported that identical results for human apo AI kinetics were obtained when either apo AI or HDL was used as the tracer (30, 31). In our studies we did not

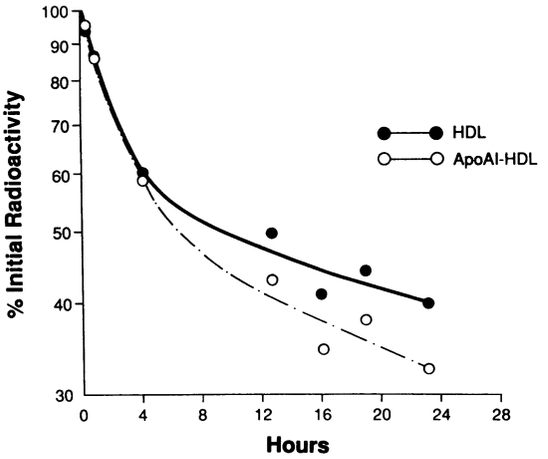

Figure 4. Plasma decay of ^{125}I-TC apo AI HDL and ^{131}I-TC HDL in the cynomolgus monkey. The tracers were prepared as described in Methods and simultaneously injected into a monkey, and the plasma decay curves were obtained.

perform simultaneous studies using HDL and apo AI tracers to address this question directly. Nevertheless, our control studies using different tracers did not demonstrate marked differences in FCR using different apo AI tracers, especially when compared to the differences seen with LPL inhibition. The increased catabolism of TC-apo AI might indicate that in this monkey apo AI FCR was greater than that of HDL apolipoprotein. Alternatively, the increased rate of clearance of TC-apo AI could indicate that the protein was altered during the labeling process.

Despite the relatively minor differences in plasma turnover, tissue uptake of the TC-apo AI and TC-HDL in the monkey was dramatically different. A comparison of the uptake of the two labels into several major tissues is shown in Fig. 5. The ratios of label per gram in kidney/liver using labeled apo AI and labeled intact HDL were, respectively, 11.6 and 1.6. Because over 85% of the TC-labeled protein in the HDL

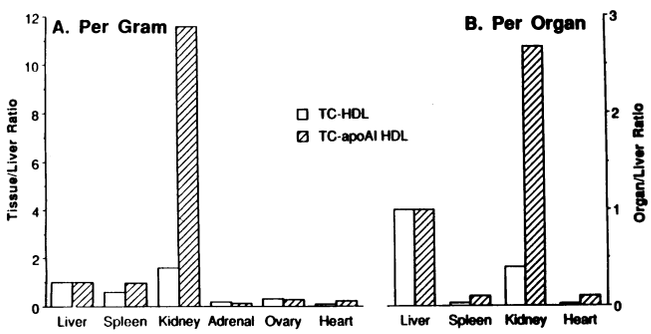

Figure 5. Comparison of the tissue sites of catabolism of ^{125}I-TC apo AI HDL and ^{131}I-TC HDL. ^{125}I-TC apo AI HDL and ^{131}I-TC HDL were prepared as described in Methods and simultaneously injected into a cynomolgus monkey. After 24 h the animal was killed by pentobarbital overdose followed by exsanguination and the tissues and carcass were perfused with saline to remove any residual blood. The amounts of each tracer in the tissues were determined and the relative amount of each tracer per organ was determined. The data shown compared the ratio of organ uptakes to liver, which is set at 1.

was apo AI (as demonstrated by PAGE), these data imply that renal uptake of TC labeled apo AI is greater than TC-labeled apo AI on intact HDL. Furthermore, TC labeled apo AI exchanged onto HDL does not appear to be a good tracer to study tissue uptake of HDL protein. A similar observation was reported in rats by Glass et al. (9). To eliminate the label which was rapidly cleared by the kidney, these investigators used radioiodinated TC-apo AI HDL obtained after injection of the initial labeled HDL into a donor rat. Because this technique requires the sacrifice of donor animals, it was not a feasible approach to use in primate studies and all subsequent studies were performed using TC-labeled intact HDL.

We next tested whether major differences occurred in the distribution of labeled TC, injected as TC-HDL, between organs from control and LPL-inhibited monkeys. The amounts of radioactivity and the percentages of the injected dose in organs obtained from six monkeys (three control and three LPL-inhibited) after injection of ^{125}I-TC-HDL are given in Tables V and VI. The major organs responsible for clearance of HDL apolipoprotein were the liver and kidney and significant amounts of label were also found in the spleen. Steroid producing glands (ovary and adrenal) are less important for HDL catabolism in the monkey than the rat (9), which uses HDL as the major plasma source of cholesterol for steroidogenesis. In the control studies, the extraction of label per gram of tissue was slightly, 1.4-fold, greater for the kidney than the liver. Because of the larger size of the liver, per organ, the removal of tracer by the kidney was only 35% of the amount of tracer removed by the liver.

The three studies performed using paired control and experimental monkeys showed similar results; renal uptake of tracer was greater in LPL-inhibited monkeys and liver uptake was unchanged. The extraction of label per gram of tissue was ~ 1.8-fold greater in kidneys of LPL inhibited monkeys and kidney clearance of label increased to 64% of that of liver. To best illustrate this difference in organ uptake with LPL inhibition, the ratio of the amount of label per gram wet weight of kidney versus gram wet weight of liver in each of the three pairs of animals are shown in Fig. 6. Although there may have been some differences in the uptake by other organs, e.g., less uptake by the heart and more by the spleen during LPL inhibition, the percent difference in uptake of the injected tracer by those organs (Table VI) was too small to account for changes in plasma HDL protein FCR. The liver removed similar amounts of label in normal and LPL-inhibited monkeys. Thus, the increase in HDL protein FCR during LPL inhibition was, at least in part, due to increased removal of apoprotein by the kidney.

At the conclusion of the study, less label remained in the plasma of the LPL-inhibited monkeys (22.5% vs. 39%, Table VI), consistent with the increased HDL protein FCR that occurred during LPL inhibition (see Table IV). For reasons which are unclear, the total recovery of tracer in the organs from the LPL-inhibited monkeys was lower than that in the

Table V. Tissue Sites of HDL Protein Catabolism Assessed by Recovery of Radioactive TC, Injected as ^{125}I-TC-HDL, in Organs from Control and LPL-inhibited Monkeys

Organ	Uptake		Uptake	
	Control	LPL inhibited	Control	LPL inhibited
	cpm/g × 10³		*cpm/organ × 10³*	
Liver	22.3±1.5	21.5±1.4	2,118±94	2,057±89
Kidney	31.9±2.1	57.3±5.7	752±90	1,314±160
Spleen	19.7±3.3	15.6±0.87	157±29	193±25
Adrenal	4.6±0.43	11.4±2.5	8.9±0.8	15±4
Heart	2.4±0.25	1.8±0.35	40±0.6	25±5
Pancreas	1.1±0.13	0.9±0.03	8.5±1.9	9±0.42
Small Intestine	0.86±0.19	1.3±0.43		
Lung	2.8±0.65	1.4±0.33		
Muscle	0.53±0.13	0.24±0.02		
Fat	0.75±0.19	1.5±0.83		
Skin	1.03±0.19	1.1±0.51		

Radioactivity (average ± SEM) recovered in each organ was calculated by multiplying the uptake per gram by the total wet weight of each organ. To correct for variability in the amounts of tracer injected, data were normalized to an injection of 5 μCi. Studies were performed using paired animals and the data presented are the averages obtained from three control and three LPL-inhibited monkeys.

Table VI. Percentage of the Injected Labeled TC, Injected as TC-HDL, Recovered in Plasma and Tissues of Normal and LPL-inhibited Monkeys

Site	Control	LPL inhibited
	%	
Liver	21.7±1.5	20.5±0.8
Kidney	8.8±0.6	13.4±1.7
Spleen	1.2±0.3	1.9±0.3
Heart	0.4±0.07	0.3±0.05
Adrenal	0.1±0.03	0.15±0.04
Pancreas	0.1±0.03	0.1±0.002
Plasma	39.0±2.5	22.5±2.2

The amount of tracer (average ± SEM) recovered in each organ was divided by the total injected dose. Percentage of dose remaining in plasma was determined by the difference between the amount of radioactivity in the initial (2-min) and final (24-h) blood samples. Studies were performed using paired animals and the data presented are the averages obtained from three control and three LPL-inhibited monkeys.

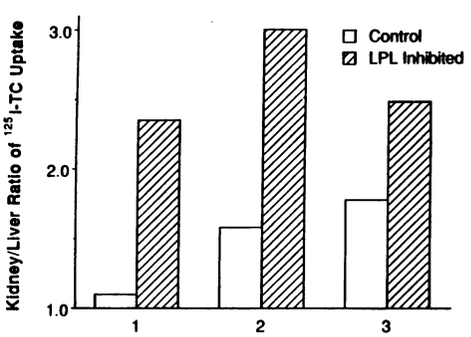

Figure 6. Relative uptake of TC HDL by kidney and liver of control and LPL-inhibited monkeys. The ratios of radioactivity per gram of kidney/liver 24 h after injection of ^{125}I-LPL–TC HDL are shown. The studies were performed using three pairs of animals, monkeys 6–11, and the differences in the ratio between control and LPL-inhibited monkeys are significant ($P < 0.05$) by paired t test.

control monkeys. It is possible that more tracer was eliminated in the urine or stool of these animals or that more label was taken up by tissues that were not sampled, such as bone marrow or brain. Even though the absolute uptake of tracer varied, the percents of non-TCA-precipitable counts, which represent TC associated with products of the intracellular degradation of its carrier protein, were identical in control and experimental monkeys, averaging 37% in the liver and 48% in the kidney. Both the lower amount of organ uptake and reduced amount of non-TCA-precipitable radioactivity compared to the rat studies (9) likely resulted from the shorter (24 vs. 48 h) duration of our studies.

Discussion

The risk of the development of coronary heart disease in humans is strongly related to the level of circulating HDL cholesterol (1–3). The factors regulating HDL cholesterol levels are, therefore, of fundamental importance both for lipoprotein physiology and for understanding the relationship of this lipoprotein to coronary heart disease. A major determinant of HDL cholesterol levels and the number of circulating HDL particles is the plasma levels of apo AI, the major structural protein of HDL. Uncommon genetically determined defects in the production of apo AI result in low levels of HDL (32). In most humans on a standard diet, however, kinetic studies of apo AI turnover suggest that the plasma levels of apo AI and HDL cholesterol are correlated with the apo AI FCR (33, 34). This suggests that important events responsible for regulating apo AI and HDL cholesterol levels occur within the circulation and are not due to variation in the synthetic rate of the apo AI molecule. By contrast, some dietary variations in humans (35, 36) or other primates (37) appear to alter apo AI production.

Our studies demonstrate that inhibition of LPL activity in monkeys results in increased fractional catabolism of apo AI. This was demonstrated in studies using apo AI exchanged onto HDL as the tracer, as well as using HDL which was labeled intact and which contained > 85% of the label on apo AI. Apo AI is present in plasma in three interchangeable pools: (*a*) on HDL, (*b*) on less dense lipoproteins, and (*c*) in the nonlipoprotein-containing fraction of plasma. Because all three pools are interchangeable, the FCR for apo AI is identical whether data obtained from plasma or isolated lipoproteins is used. This was true in our studies. Because data obtained using whole plasma do not have potential problems of incomplete recovery of lipoproteins during ultracentrifugation, the data presented were from plasma.

The levels of HDL apo AI protein are determined by three separate processes: (*a*) protein synthesis, (*b*) fractional removal of apo AI from the plasma, and (*c*) distribution of apo AI between HDL and other plasma components. Lipolysis of TG-rich lipoproteins leads to transfer of lipid and apoproteins to HDL, producing larger HDL. This process results in a redistribution of apo AI from nascent chylomicrons and VLDL to HDL. LPL inhibition blocks this process which may account for an increased proportion of apo AI associated with TG-rich lipoproteins. Alternatively, hypertriglyceridemia alone, without an obvious abnormality in LPL activity, may lead to transfer of greater amounts of apo AI to TG-rich lipoproteins. Our tracer kinetic studies measure apo AI synthetic rate as the appearance of apo AI in the plasma. There is no evident mechanism whereby the plasma distribution of apo AI should affect its synthetic rate. Therefore, it is not surprising that in this study LPL inhibition did not dramatically change HDL apolipoprotein or apo AI synthetic rates.

Why is HDL apolipoprotein FCR increased during LPL inhibition? Several possible changes in the composition of HDL or alterations in the distribution of apo AI in the plasma could be involved. (*a*) Increased removal of HDL apolipoproteins could follow their delayed egress from or increased transfer to the expanded pool of TG-rich lipoproteins (VLDL and chylomicrons). Even during LPL inhibition which prevents or decreases the conversion of TG-rich lipoproteins into remnants, these lipoproteins may be removed more rapidly from the circulation than HDL. TG-rich lipoproteins are primarily removed from the circulation by the liver, therefore, during LPL inhibition increased clearance of plasma apo AI along with TG-rich lipoproteins may lead to greater hepatic uptake of apo AI. (*b*) Decreased transfer of surface apoproteins from TG-rich lipoproteins onto HDL might lead to more rapid clearance of the HDL from the circulation. The most important of these transferable apoproteins may be apo CIII, which decreases hepatic uptake of HDL in perfused livers (38). Apo CIII-deficient HDL are also likely to be taken up in greater amounts by the liver. The lack of any observed increase in HDL apolipoprotein uptake by the liver during LPL inhibition makes these two possibilities unlikely.

A third possible reason for the accelerated HDL apolipoprotein clearance during LPL inhibition relates to changes in the composition of HDL. During LPL inhibition, HDL become TG enriched which should make it a better substrate for lipolytic enzymes, e.g., HTGL (39, 40). As the HDL core lipids, especially TG, are hydrolyzed, smaller lipid-poor particles or apo AI unassociated with lipid result. These forms of apo AI may be cleared by the kidney. Alternatively, changes in HDL lipid composition could alter the affinity of apo AI association with HDL. An indication of a change in apo AI association with HDL would be an increase in apo AI tracer found in the non-lipoprotein fraction of plasma after ultracentrifugation. On average, during LPL inhibition more tracer was found in the nonlipoprotein fraction of plasma and more HDL apolipoprotein uptake occurred in the kidney. Therefore, we believe that this third mechanism is likely to be involved in the increase in HDL apolipoprotein FCR.

Using ^{125}I-TC-HDL, a label which is trapped intracellularly after its clearance from the plasma and degradation of its carrier protein in the lysosome, we showed that increased HDL catabolism during LPL inhibition was due, at least in part, to a greater clearance of HDL protein by the kidney. Increased HDL catabolism by the kidney may occur via the generation of more delipidated apo AI (free apo AI). Glass et al. (9) reported that much of the apo AI exchanged onto HDL is cleared from the plasma by the rat kidney; our data using ^{125}I-TC-apo AI demonstrated a similar role for the kidney in apo AI catabolism in the monkey. These authors also showed that HDL lipid was primarily cleared by the liver. This suggested that some apo AI cleared by the kidney was apoprotein which contained little or no associated cholesteryl ester.

Does lipid-poor apo AI which can be filtered by the kidney ever appear in plasma? Lipid-poor apo AI has been found in the plasma of patients with renal failure (41). Furthermore,

large amounts of apo AI have been measured in the urine of patients with nephrotic syndrome (42), who presumably cannot reabsorb all the filtered apo AI. Roheim et al. (43) detected lipoprotein-free apo AI in lymph but not plasma of dogs. Thus, when this free apo AI enters the circulation, it must either be rapidly associated with lipid or cleared quickly from the plasma. Small amounts of apo AI are known to dissociate from HDL during ultracentrifugation. Melchior and Castle (28) have recently demonstrated that greater amounts of apo AI are unassociated with HDL in hyperlipidemic monkey plasmas. Similarly, Ishikawa and Fidge (44) showed that injection of Triton into rats resulted in more apo AI in the $d > 1.21$ g/ml fraction of plasma and increased fractional catabolism of this apoprotein. Free apo AI might also be generated during hydrolysis of TG-rich particles with apo AI on their surface.

An obvious question is whether alterations in HDL composition without concomitant changes in LPL activity, will lead to changes in HDL catabolism. As an initial approach to this question, iodinated normal and hypertriglyceridemic human HDL were injected into normolipidemic rats and monkeys. The turnover of these HDL were not different (data not shown). Remodeling of the lipid in the core of the injected lipoproteins should not have occurred in the studies in the rat, due to the lack of cholesteryl ester transfer protein. The labeled apo AI might have, however, equilibrated with nonlabeled rat apo AI (45). Such an equilibration would negate any differences in catabolism of the two HDL preparations. Hence, increased clearance of different HDL preparations appears to be difficult to demonstrate in vivo and will require other methods.

Although the hypertriglyceridemia produced in the monkeys was caused by immunological blockade of LPL, we contend that this is a reasonable model for many examples of hypertriglyceridemia and low HDL cholesterol levels in humans. Variations of LPL activity in humans are associated with changes in lipoproteins which are consistent with our findings in the monkey. This is best illustrated by the positive correlation between the activity of LPL and the rate of postprandial TG catabolism and HDL cholesterol levels (11, 46). Thus, HDL cholesterol levels in general are an index of the activity of LPL in vivo.

Increases in apo AI FCR owing to abnormal HDL composition might occur in normolipidemic or hypertriglyceridemic humans who have low LPL activity. In some people with low levels of HDL, fasting hypertriglyceridemia is not present. Nontheless, these individuals may have elevated plasma TG levels much of the day when they are in a postprandial state. During hypertriglyceridemia the neutral lipid composition of HDL is altered by elevated levels of circulating TG as TG is exchanged for HDL cholesteryl ester (47, 48). Thus, TG-rich HDL, comparable to that found in LPL-inhibited monkey plasma, may be formed. The increase in apo AI FCR and the low plasma apo AI levels in hypertriglyceridemic humans (33) may be due to mechanisms similar to those observed during LPL inhibition in our studies.

LPL inhibition and the ensuing hypertriglyceridemia did not appear to affect LDL FCR. Hypertriglyceridemic humans often have an increased LDL FCR (49, 50) and a metabolic defect that results in overproduction of VLDL and apoprotein B (the major structural protein of both VLDL and LDL) (51–53). Despite the expanded pool of VLDL which may compete with LDL for binding to the LDL receptor (54), LDL FCR is increased, suggesting that these patients may have a defect which increases LDL receptor activity in addition to or as a result of the regulatory defect in apo B production. In our studies LDL was isolated from normolipidemic monkeys and the same radioiodinated LDL preparations were injected into control and LPL inhibited monkeys. This protocol allowed us to evaluate specifically the way the host handled LDL rather than assessing differences which may be due to variations in LDL composition. LDL FCR was similar in both control and LPL-inhibited monkeys, suggesting that acute LPL inhibition did not alter processes, such as the activity of the LDL receptor, responsible for LDL catabolism. This interpretation requires several assumptions regarding the injection of control tracer LDL into LPL-inhibited monkeys. Very rapid exchange of cholesteryl esters occurs in monkeys (55, 56). After injection, the tracer LDL may have been converted to LDL with a composition which closely resembled that of the autologus LDL. Thus, in the LPL-inhibited monkeys the LDL FCR may actually reflect the clearance of TG-rich LDL rather than normal LDL in these monkeys.

Our studies provide a model which suggests that variations in apo AI levels between humans due to variations in apo AI FCR may be a consequence of differences in LPL activity and rates of TG-rich lipoprotein lipolysis. In this regard, Magill et al. (57) reported that human apo AI FCR is inversely correlated with VLDL apo B FCR and adipose tissue LPL activity. Interventions that increase LPL activity, such as chronic exercise (58), insulin treatment of diabetes mellitus (59), or treatment with fibric acid derivatives (60), would be expected to (*a*) lower plasma TG, (*b*) increase plasma apo AI levels, and (*c*) increase HDL cholesterol levels. Variations in LPL activity may account for a significant portion of the variability in levels of HDL cholesterol and apolipoprotein and for mechanisms which affect atherogenic risk by alteration in HDL levels (61).

Acknowledgments

The authors wish to thank Dr. Henry Ginsberg for many helpful comments, Dr. Sulli Popilskis for assistance with the animal surgery, Dr. Charles Bisgaier for performing the apo AI measurements, Dr. DeWitt S. Goodman for reviewing the manuscript, and Ms. Linda Martinez for her secretarial assistance.

These studies were supported by grants HL-21006 (SCOR), a grant-in aid from the American Heart Association and Bristol-Myers, and research support from the Council for Tobacco Research, USA, Inc. Dr. Goldberg is the recipient of an Established Fellowship from the American Heart Association, New York City Affiliate.

References

1. Miller, G. J. 1980. High density lipoproteins and atherosclerosis. *Annu. Rev. Med.* 31:97–108.

2. Gordon, T., W. P. Castelli, M. C. Hjortland, W. B. Kannel, and T. R. Dawber. 1977. High density lipoprotein as a protective factor against coronary heart disease: the Framingham Study. *Am. J. Med.* 62:707–714.

3. Holmes, D. R., Jr., L. R. Elveback, R. L. Frye, B. A. Kottke, and R. D. Ellefson. 1981. Association of risk factor variables and coronary artery disease documented with angiography. *Circulation.* 63:293–299.

4. Eisenberg, S. 1984. High density lipoprotein metabolism. *J. Lipid Res.* 25:1017–1058.

5. Garfinkel, A. S., and M. C. Schotz. 1987. Lipoprotein lipase. *In*

Plasma Lipoproteins. A. M. Gotto, Jr., editor. Elsevier Science Publishing Co., Inc., New York. 335–357.

6. Oram, J. F., E. A. Brinton, and E. L. Bierman. 1983. Regulation of high density lipoprotein receptor activity in cultured human skin fibroblasts and human arterial smooth muscle cells. *J. Clin. Invest.* 72:1611–1621.

7. Rifici, V. A., and H. A. Eder. 1984. A hepatocyte receptor for high density lipoproteins specific for apolipoprotein AI. *J. Biol. Chem.* 259:13814–13818.

8. Glass, C., R. C. Pittman, M. Civen, and D. Steinberg. 1985. Uptake of high density lipoprotein-associated apoprotein AI and cholesterol esters by 16 tissues of the rat in vivo and by adrenal cells and hepatocytes in vitro. *J. Biol. Chem.* 260:744–750.

9. Glass, C. K., R. C. Pittman, G. A. Keller, and D. Steinberg. 1983. Tissue sites of degradation of apoprotein AI in the rat. *J. Biol. Chem.* 258:7161–7167.

10. Huttunnen, L. V., C. Ehnholm, M. Kekki, and E. A. Nikkila. 1977. Postheparin plasma lipoprotein lipase and hepatic lipase in normal subjects relationship to age, sex, and triglyceride metabolism. *Adv. Exp. Med. Biol.* 82:146–148.

11. Patsch, J. R., S. Prasad, A. M. Gotto, Jr., and W. Patsch. 1987. High density lipoprotein$_2$: relationship of the plasma levels of this lipoprotein species to its composition, to the magnitude of postprandial lipidemia, and to the activities of lipoprotein lipase and hepatic lipase. *J. Clin. Invest.* 80:341–347.

12. Kekki, M. 1980. Lipoprotein lipase action determining plasma high density lipoprotein cholesterol level in adult normolipaemics. *Atherosclerosis.* 37:143–150.

13. Goldberg, I. J., N. A. Le, H. N. Ginsberg, J. R. Paterniti, and W. V. Brown. 1983. Metabolism of apolipoprotein B in the cynomolgus monkey. *Am. J. Physiol.* 244:E196–E201.

14. Goldberg, I. J., N. A. Le, H. N. Ginsberg, R. M. Krauss, and F. T. Lindgren. 1988. Lipoprotein metabolism during acute inhibition of lipoprotein lipase in the cynomolgus monkey. *J. Clin. Invest.* 81:561–568.

15. Goldberg, I. J., N. A. Le, J. R. Paterniti, Jr., H. N. Ginsberg, F. T. Lindgren, and W. V. Brown. 1982. Lipoprotein metabolism during acute inhibition of hepatic triglyceride lipase in the cynomolgus monkey. *J. Clin. Invest.* 70:1184–1192.

16. Assmann, G. H. Schriewer, G. Schmitz, and E. O. Hagele. 1983. Quantitation of high-density-lipoprotein cholesterol by precipitation with phosphotungstic acid/$MgCl_2$. *Clin. Chem.* 29:2026–2030.

17. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* 34:1345–1353.

18. MacFarlane, A. S. 1958. Efficient trace labeling of proteins with iodine. *Nature (Lond.).* 182:53.

19. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoprotein proteins. 1. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* 260:212–221.

20. Polacek, D., C. Edelstein, and A. M. Scanu. 1981. Rapid fractionation of human high density apolipoproteins by high performance liquid chromatography. *Lipids.* 16:927–929.

21. Dell, R. B., R. Sciacca, K. Lieberman, D. B. Case, and P. J. Cannon. 1973. A weighted least-squares technique for the analysis of kinetic data and its application to the study of ^{133}Xenon washout in dogs and in man. *Circ. Res.* 21:71–84.

22. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:267–275.

23. Bisgaier, C. L., O. P. Sachdev, E. S. Lee, K. J. Williams, C. B. Blum, and R. M. Glickman. 1987. Effect of lecithin:cholesterol acyltransferase on distribution of apolipoprotein A-IV among lipoproteins of human plasma. *J. Lipid Res.* 28:693–703.

24. Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.

25. Pittman, R. C., and C. A. Taylor, Jr. 1986. Methods for assessment of tissue sites of lipoprotein degradation. *Methods Enzymol.* 129:612–628.

26. Huettinger, M., W. J. Schneider, Y. K. Ho, J. L. Goldstein, and M. S. Brown. 1984. Use of monoclonal anti-receptor antibodies to probe the expression of the low density lipoprotein receptor in Watanabe heritable hyperlipidemic rabbits. *J. Clin. Invest.* 74:1017–1026.

27. Parks, J. S., and L. L. Rudel. 1979. Isolation and characterization of high density lipoprotein apoproteins in the non-human primate (vervet). *J. Biol. Chem.* 254:6716–6723.

28. Melchior, G. W., and C. K. Castle. 1989. Apo AI metabolism in the cynomolgus monkey: identification and characterization of β-migrating pools. *Arteriosclerosis.* 9:470–478.

29. Shephard, J., C. J. Pachard, A. M. Gotto, Jr. and O. D. Taunton. 1978. A comparison of two methods to investigate the metabolism of human apolipoproteins A-I and A-II. *J. Lipid. Res.* 19:656–661.

30. Schaefer, E. J., L. A. Zech, L. L. Jenkins, T. J. Bronzert, E. A. Rubalcaba, F. T. Lindgren, R. L. Aamodt, H. B. Brewer, Jr. 1982. Human apolipoprotein A-I and A-II metabolism. *J. Lipid. Res.* 23:850–862.

31. Malmendier, C. L., C. Delcroix, and J. P. Ameryckx. 1983. In vivo metabolism of human apoprotein A-I-phospholipid complexes: comparison with human high density lipoprotein-apoprotein A-I metabolism. *Clin. Chim. Acta.* 131:201–210.

32. Karathanasis, S. K., J. McPherson, V. I. Zannis, and J. L. Breslow. 1983. An inherited polymorphism in the human apolipoprotein AI gene locus related to the development of atherosclerosis. *Nature (Lond.).* 301:718–720.

33. Fidge, N., P. Nestel, T. Ishikawa, M. Reardon, and T. Billington. 1985. Turnover of apoproteins AI and AII of high density lipoprotein and the relationship to other lipoproteins in normal and hyperlipidemic individuals. *Metab. Clin. Exp.* 29:643–653.

34. Brinton, E. A., S. Eisenberg, and J. L. Breslow. 1989. Elevated high density lipoprotein cholesterol levels correlate with decreased apolipoprotein A-I and A-II fractional catabolic rate in women. *J. Clin. Invest.* 84:262–269.

35. Shepard, J., C. J. Packard, J. R. Patsch, A. M. Gotto, Jr., and O. D. Taunton. 1978. Effects of dietary polyunsaturated and saturated fat on properties of high density lipoproteins and the metabolism of apolipoprotein AI. *J. Clin. Invest.* 61:1582–1592.

36. Brinton, E. A., S. Eisenberg, and J. L. Breslow. 1990. A low-fat diet decreases high density (HDL) cholesterol levels by decreasing HDL apolipoprotein transport rate. *J. Clin. Invest.* 85:144–151.

37. Sorci-Thomas, M., M. M. Prack, N. Dashti, F. Johnson, L. L. Rudel, and D. L. Williams. 1988. Apolipoprotein (APO) AI production and mRNA abundance explain plasma ApoAI and high density lipoprotein differences between two nonhuman primate species with high and low susceptibilities to diet-induced hypercholesterolemia. *J. Biol. Chem.* 263:5183–5189.

38. Quarfordt, S., J. Hanks, R. S. Jones, and F. Shelburne. 1980. The uptake of high density lipoprotein cholesteryl ester in the perfused rat liver. *J. Biol. Chem.* 255:2934–2937.

39. Musliner, T. A., P. N. Herbert, and M. J. Kingston. 1979. Lipoprotein substrates of lipoprotein lipase and hepatic triglycerol lipase from human postheparin plasma. *Biochim. Biophys. Acta.* 575:277–288.

40. Patsch, J. R., S. Prasad, A. M. Gotto, Jr., and G. Bengtsson-Olivecrona. 1984. Postprandial lipemia: a key for the conversion of high density lipoprotein$_2$ into high density lipoprotein$_3$ by hepatic lipase. *J. Clin. Invest.* 74:2017–2023.

41. Neary, R. H., and E. Gowland. 1988. The effect of renal failure and haemodialysis on the concentration of free apolipoprotein AI in serum and the implications for the catabolism of high density lipoproteins. *Clin. Chim. Acta.* 171:239–246.

42. Gitlin, D., D. G. Cornwall, D. Nakasato, J. L. Oncley, W. L. Hughes, Jr., and C. A. Janeway. 1958. Studies on the metabolism of

plasma proteins in the nephrotic syndrome II: the lipoproteins. *J. Clin. Invest.* 37:172–184.

43. Roheim, P. S., C. H. Sloop, J. Goudey-Lefevre, and M. Lefevre. 1988. Evidence for the peripheral formation of lipoprotein-free apo AI with slow pre-β electrophoretic mobility. 8th International Symposium on Atherosclerosis. Tekno Press, Rome. 781.

44. Ishikawa, T., and N. Fidge. 1979. Changes in the concentration of plasma lipoproteins and apoproteins following the administration of Triton WR 1339 to rats. *J. Lipid Res.* 20:254–264.

45. Shepard, J., J. R. Patsch, C. J. Packard, A. M. Gotto, Jr., and O. D. Taunton. 1978. Dynamic properties of human high density lipoprotein apoproteins. *J. Lipid Res.* 19:383–383.

46. Patsch, J. R., J. B. Karlin, L. W. Scott, L. C. Smith, and A. M. Gotto, Jr. 1983. Inverse relationship between blood levels of high density lipoprotein subfraction 2 and magnitude of postprandial lipemia. *Proc. Natl. Acad. Sci. USA.* 80:1449–1453.

47. Groot, P. H. E., and L. M. Scheek. 1984. Effects of fat ingestion on high density lipoprotein profiles in human sera. *J. Lipid. Res.* 25:684–692.

48. Tall, A., D. Sammett, and E. Granot. 1986. Mechanisms of enhanced cholesteryl ester transfer from high density lipoproteins to apolipoprotein B-containing lipoproteins during alimentary lipemia. *J. Clin. Invest.* 77:1163–1172.

49. Kissebah, A. H., S. Alfarsi, and D. J. Evans. 1984. Low density lipoprotein metabolism in familial combined hyperlipidemia: mechanism of the multiple lipoprotein phenotypic expression. *Arteriosclerosis.* 4:614–624.

50. Vega, G. L., W. F. Beltz, and S. M. Grundy. 1985. Low density lipoprotein metabolism in hypertriglyceridemic and normolipidemic patients with coronary heart disease. *J. Lipid Res.* 26:115–126.

51. Berman, M., M. Hall, R. I. Levy, S. Eisenberg, D. W. Bilheimer, R. D. Phair, and R. H. Goebel. 1978. Metabolism of apo B and apo C lipoproteins in man: kinetic studies in normal and hyperlipoproteinemic subjects. *J. Lipid. Res.* 19:38–55.

52. Chait, A., J. J. Albers, and J. D. Brunzell. 1980. Very low density lipoprotein overproduction in genetic forms of hypertriglyceridemia. *Eur. J. Clin. Invest.* 10:17–22.

53. Ginsberg, H. N., N. A. Le, and J. C. Gibson. 1985. Regulation of the production and catabolism of plasma low density lipoproteins in hypertriglyceridemic subjects. *J. Clin. Invest.* 75:614–623.

54. Vega, G. L., C. East, and S. M. Grundy. 1988. Lovastatin therapy in familial dysbetalipoproteinemia: effects on kinetics of apolipoprotein B. *Atherosclerosis.* 70:131–143.

55. Goldberg, I. J., R. S. Rosenfeld, I. Paul, and B. Leeman. 1986. Generation of plasma free cholesterol from circulating lipoprotein associated cholesteryl ester. *Am. J. Physiol.* 250:E265–E268.

56. Thomas, M. S., and L. L. Rudel. 1983. [^{3}H]Cholesteryl ester labeling and transfer among human and nonhuman primate plasma lipoproteins. *Anal. Biochem.* 130:215–222.

57. Magill, P., S. N. Rao, N. E. Miller, A. Nicoll, J. Brunzell, J. St. Hilaire, and B. Lewis. 1982. Relationship between the metabolism of high-density and very-low-density lipoprotein in man: studies of apolipoprotein kinetics and adipose tissue lipoprotein lipase activity. *Eur. J. Clin. Invest.* 12:113–120.

58. Herbert, P. N., D. N. Bernier, E. M. Cullinane, L. Edelstein, M. A. Kantor, and P. D. Thompson. 1984. High density lipoprotein metabolism in runners and sedentary men. *JAMA (J. Am. Med. Assoc.)* 252:1034–1037.

59. Taskinen, M.-R., and E. A. Nikkila. 1979. Lipoprotein lipase activity of adipose tissue and skeletal muscle in insulin-deficient human diabetes. *Diabetologia.* 17:351–356.

60. Boberg, J., M. Boberg, R. Gross, S. Grundy, J. Augustin, and W. V. Brown. 1977. The effect of treatment with clofibrate on hepatic triglyceride and lipoprotein lipase activities of post heparin plasma in male patients with hyperlipoproteinemia. *Atherosclerosis.* 27:499–503.

61. Frick, M. H., O. Elo, K. Haapa, O. P. Heinonen, P. Heinsalmi, P. Helo, J. K. Huttunen, P. Kaitaniemi, P. Koskinen, V. Manninen, et al. 1987. Helsinki Heart Study: Primary-Prevention Trial with gemfibrozil in middle-aged men with dyslipidemia. *N. Engl. J. Med.* 317:1237–1245.