Mechanism of Hypertriglyceridemia in Human Apolipoprotein (Apo) CIII Transgenic Mice

Diminished Very Low Density Lipoprotein Fractional Catabolic Rate Associated with Increased Apo CIII and Reduced Apo E on the Particles

Katriina Aalto-Setälä, Edward A. Fisher,* Xiaoli Chen,* Tova Chajek-Shaul, Tony Hayek, Rudolf Zechner,‡ Annemarie Walsh, Rajasekhar Ramakrishnan,§ Henry N. Ginsberg,§ and Jan L. Breslow

*Laboratory of Biochemical Genetics and Metabolism, The Rockefeller University, New York 10021; *Department of Physiology and Biochemistry, The Medical College of Pennsylvania, Philadelphia, Pennsylvania 19129; ‡Institute of Medical Biochemistry, Karl Franzens University Graz, A-8010 Graz, Austria; and §Department of Medicine, College of Physicians and Surgeons, Columbia University, New York 10032*

Abstract

Hypertriglyceridemia is common in the general population, but its mechanism is largely unknown. In previous work human apo CIII transgenic (HuCIIITg) mice were found to have elevated triglyceride levels. In this report, the mechanism for the hypertriglyceridemia was studied. Two different HuCIIITg mouse lines were used: a low expressor line with serum triglycerides of ~ 280 mg/dl, and a high expressor line with serum triglycerides of ~ 1,000 mg/dl. Elevated triglycerides were mainly in VLDL. VLDL particles were 1.5 times more triglyceride-rich in high expressor mice than in controls. The total amount of apo CIII (human and mouse) per VLDL particle was 2 and 2.5 times the normal amount in low and high expressors, respectively. Mouse apo E was decreased by 35 and 77% in low and high expressor mice, respectively. Under electron microscopy, VLDL particles from low and high expressor mice were found to have a larger mean diameter, 55.2±16.6 and 58.2±17.8 nm, respectively, compared with 51.0±13.4 nm from control mice. In in vivo studies, radiolabeled VLDL fractional catabolic rate (FCR) was reduced in low and high expressor mice to 2.58 and 0.77 pools/h, respectively, compared with 7.67 pools/h in controls, with no significant differences in the VLDL production rates. In an attempt to explain the reduced VLDL FCR in transgenic mice, tissue lipoprotein lipase (LPL) activity was determined in control and high expressor mice and no differences were observed. Also, VLDLs obtained from control and high expressor mice were found to be equally good substrates for purified LPL. Thus excess apo CIII in HuCIIITg mice does not cause reduced VLDL FCR by suppressing the amount of extractable LPL in tissues or making HuCIIITg VLDL a bad substrate for LPL. Tissue uptake of VLDL was studied in hepatoma cell cultures, and VLDL from transgenic mice was found to be taken up much more slowly than control VLDL ($P < 0.0001$), indicating that HuCIIITg VLDL is not well recognized by lipoprotein receptors. Additional in vivo studies with Triton-treated mice showed increased VLDL triglyceride, but not apo B, production in the HuCIIITg mice compared with controls. Tissue culture studies with primary hepatocytes showed a modest increase in triglyceride, but not apo B or total protein, secretion in high expressor mice compared with controls. In summary, hypertriglyceridemia in HuCIIITg mice appears to result primarily from decreased tissue uptake of triglyceride-rich particles from the circulation, which is most likely due to increased apo CIII and decreased apo E on VLDL particles. The HuCIIITg mouse appears to be a suitable animal model of primary familial hypertriglyceridemia, and these studies suggest a possible mechanism for this common lipoprotein disorder. (*J. Clin. Invest.* 1992. 90:1889–1900.) Key words: lipoprotein lipase • fractional catabolic rate • production rate • primary hepatocytes • free fatty acids

Introduction

Hypertriglyceridemia is a common finding in the general population. Although it can be caused by many factors, including dietary habits, alcohol intake, physical activity, medication, and different diseases (for review, see reference 1), it is clear that a relatively large number of individuals have a genetic tendency to hypertriglyceridemia. A few of these people have been shown to have mutations in the lipoprotein lipase (LPL)[1] gene, the protein product of which hydrolyzes triglycerides in chylomicrons and VLDL (2). Others have a defect in the apo CII gene, which codes for a cofactor protein in the LPL reaction (3). However, in the vast majority of subjects with primary hypertriglyceridemia, the genetic defect is still unknown.

Apo CIII is a 79 amino acid glycoprotein accounting in humans for ~ 50% of VLDL and ~ 2% of HDL proteins (4). The level of apo CIII is known to correlate with serum triglyceride levels (5–7) and a DNA polymorphism in the 3′ noncoding region of the apo CIII gene has been associated with hypertriglyceridemia in several populations (for review, see reference 8). However, the role of apo CIII in triglyceride metabolism is not well understood. It has been postulated that apo CIII might inhibit LPL (9–11) and also that it might inhibit the uptake of triglyceride-rich lipoproteins and their remnants by the liver

Address reprint requests to Dr. J. L. Breslow, Box H29, The Rockefeller University, 1230 York Avenue, New York, NY 10021.

Received for publication 11 February 1992 and in revised form 12 May 1992.

J. Clin. Invest.
© The American Society for Clinical Investigation, Inc.
0021-9738/92/11/1889/12 $2.00
Volume 90, November 1992, 1889–1900

1. *Abbreviations used in this paper:* ANOVA, analysis of variance; FCR, fractional catabolic rate; HuCIIITg, human apolipoprotein CIII transgenic; LPL, lipoprotein lipase; LRP, LDL receptor-related protein; PR, production rate.

(12, 13). Transgenic mouse lines expressing the human apo CIII gene were created and found to have elevated triglyceride concentrations (14). This confirms an important role for apo CIII in triglyceride metabolism in vivo and makes it possible to study the function of apo CIII in a living animal and to investigate possible mechanisms for hypertriglyceridemia.

In this study the mechanism causing elevated triglycerides in human apolipoprotein CIII transgenic (HuCIIITg) mice was studied. In HuCIIITg mice hypertriglyceridemia was mainly due to an increased number of VLDL particles in the circulation. In addition, VLDL particles in transgenic mice were found to have an altered composition. They contained more triglycerides and total apo CIII, but decreased apo E, apo CII, and mouse apo CIII compared with control VLDL. In vivo studies revealed that VLDL particles are cleared much more slowly from plasma in transgenic animals than in controls. In HuCIIITg mice, tissue LPL activity was found to be normal, as was the action of purified LPL on VLDL isolated from transgenic mice. In hepatoma cell cultures, transgenic VLDL was found to be a poorer ligand for receptor-mediated uptake than control VLDL. The diminished catabolism of transgenic VLDL might be due to the increased apo CIII and reduced apo E on the particles. Although the major metabolic abnormality appeared to be a decrease in VLDL catabolism, in vivo studies in Triton-treated mice demonstrated an increase in triglyceride production and primary hepatocytes from transgenic mice secreted more triglycerides than control hepatocytes. These studies suggest one possible mechanism for this common dyslipidemic state in humans.

Methods

Animals. Two lines of transgenic mice (C57BL6 × CBA) expressing the human apo CIII gene were used. One line (2674) characterized by triglyceride levels of ~ 280 mg/dl was designated as low expressor and the other line with triglyceride concentrations of ~ 1,000 mg/dl was designated as high expressor. Nontransgenic littermates or F1 mice (C57BL6 × CBA) were used as controls. The animals were caged in animal rooms with alternating 12-h periods of light (7 a.m.–7 p.m.) and dark (7 p.m.–7 a.m.) with ad lib access to mouse chow diet (Purina Chow) and water. Transgenic animals were identified by rocket immunoelectrophoresis using a goat antiserum to human apo CIII that does not crossreact with mouse apo CIII (14). Both male and female mice were used at 3–5 mo of age when they weighed 20–30 g. Experiments were performed between 8 a.m. and 11 a.m. with animals fed ad lib if not otherwise indicated. Animals were anesthetized with methoxyflurane for blood collection, intravenous injections, and gastric tube feedings. Bleedings were performed from the retroorbital plexus and intravenous injections were done into the femoral vein. For liver perfusion the animals were anesthetized with 5% pentobarbital. Liver biopsies were obtained from two control and two high expressor mice. They were fixed in 2.5% glutaraldehyde and 4% OsO_4 in 0.1 M Na-cacodylic buffer (pH 7.2). After dehydration the biopsies were embedded in Epon plastic. The sliced samples were poststained with 1% uranyl acetate and Pb-citrate.

Plasma lipid analysis. Blood was drawn in the morning with the animals having free access to food. VLDL, LDL, and HDL fractions were separated by sequential ultracentrifugation (15). Total plasma, VLDL, LDL and HDL cholesterol, and triglyceride concentrations were measured enzymatically using commercial kits (#236691 and #126012, respectively; Boehringer Mannheim Corp., Indianapolis, IN). Protein concentrations in the VLDL and HDL fractions were measured after spinning the samples twice and once, respectively, using a commercial kit with BSA as the standard (Bio-Rad Laboratories, Richmond, CA).

Apolipoprotein analysis. VLDL and HDL were delipidated with acetone-ethanol (1:1) (16). To identify apolipoproteins in VLDL and HDL, 50 and 25 μg of apolipoproteins, respectively, were examined by 4–15% gradient SDS-PAGE (17). After staining in 45% methanol/10% acetic acid/0.1% Coomassie Blue R and destaining in 30% methanol/10% acetic acid the gels were scanned using an UltroScan XL Laser Densitometer (LKB Instruments, Inc., Gaithersburg, MD) and the percentages of various apolipoproteins were calculated. To determine the proportion of different apo C's, isoelectric focusing was performed. A gel with a pH range of 4–6.5 containing 6 M urea, 7.5% acrylamide, 0.2% *N,N'*-methylene-bisacrylamide, and 2% ampholines was prefocused for 1 h at +4°C at 110 V and focused for 18 h at +4°C at 250 V. After staining and destaining, the protein bands were scanned and subsequently the gel was dried. In each case four different apolipoprotein preparations were used and two to four lanes representing each preparation were loaded on gels. The average for each apolipoprotein was calculated for each preparation and the means±SD of these averages are presented in Tables II and III.

Electron microscopy. To determine the size of VLDL particles, negative staining was performed with 2% potassium phosphotungstate (pH 7.6) followed by electron microscopy (18). VLDL was obtained by ultracentrifugation from two control, two low expressor, and two high expressor transgenic mice. The average size of > 100 particles in a given area was measured in each case.

Preparation of labeled VLDL. Control and HuCIIITg mice were injected intravenously with 100 μCi of either [^{3}H]glycerol or [^{3}H]-palmitate complexed with BSA (19). Blood was collected from control or high expressor mice 45 or 60 min, respectively, after the injection (20). Serum samples were ultracentrifuged to obtain the VLDL fraction. TLC on silica gel G plates (Analtech Inc., Newark, DE) using hexane-diethylether-acetic acid solution (83:16:1) as the solvent (19) was performed to ensure that 85–90% of the label was in the triglyceride fraction before the VLDL preparations were used for further studies.

In vivo removal of labeled VLDL. 11 low and 12 high expressor HuCIIITg and 17 control mice, both male and female, were injected intravenously with 200,000 dpm of ^{3}H-labeled VLDL obtained from either high expressor HuCIIITg or control mice. The disappearance of the radiolabeled VLDL was determined from serum samples drawn 2, 5, 10, 20, 40, 75, and 120 min after the injection. 60 μl of blood was drawn each time. In a pilot study, the disappearance of the counts was compared if the lipids were extracted from the serum samples and triglyceride-bound counts were determined, or if the total plasma radioactivity was measured. Since these results did not differ from each other, total plasma radioactivity was used to represent VLDL radioactivity. The radioactivity at each time point was measured and the data were modeled by a single- or two-pool model for VLDL triglyceride kinetics with a main pool and a possible remnant pool derived entirely from the main pool (21). The two-pool model was used only if it improved the fit of the data. For each animal, the fitted curve was extrapolated back to zero time and multiplied by the plasma volume (assumed to be 33 ml/kg; see reference 22) to obtain the initial radioactivity, which, divided by the injected dose, was the recovery.

In vivo triglyceride production. Anesthetized mice were injected intravenously with 500 mg/kg of Triton WR 1339 using 15% (wt/vol) Triton solution in 0.9% NaCl (23). To determine the extent of the inhibition of the triglyceride hydrolysis by Triton WR 1339, in control experiments control and high expressor mice with serum triglyceride levels of 70–120 and 510–870 mg/dl, respectively, were given 200,000 dpm of in vivo labeled VLDL intravenously 15 min after they were given Triton intravenously. The disappearance of the labeled VLDL from serum was followed. 60-μl blood samples were drawn 2, 10, 30, 60, and 90 min after ^{3}H-VLDL injection and the radioactivity in each sample was determined. The radioactivity at 2 min, at which time mixing should be complete, was regarded as 100%. Under these conditions, VLDL clearance from plasma was essentially completely inhibited in control and transgenic mice. To investigate triglyceride production, 19 control, 10 low expressor, and 14 high expressor female and male mice were Triton-treated and 15 min thereafter they received 100

μCi of [^{3}H]glycerol intravenously. Animals were bled from the retroorbital plexus before Triton injection to determine basal serum triglyceride levels and 10, 15, 20, 30, 60, and 90 min after [^{3}H]glycerol injection to determine the appearance of labeled triglycerides in serum. Lipids were extracted from serum samples using the Folch method (24) and the amount of radioactivity in the triglyceride fraction was determined by TLC (19). Radioactivity in the total lipid fraction was determined using [^{14}C]trioleate as an internal standard and the proportion of the radioactivity in the triglyceride fraction was calculated. To determine the specific activity of hepatic triglycerides, livers were removed 60 min after glycerol injection and homogenized in chloroform-methanol (1:1) solution. The triglyceride concentration was measured using a commercial kit (#701882; Boehringer Mannheim Corp.) and the radioactivity in the triglyceride fraction was determined using TLC (19).

To determine intestinal triglyceride production, three control and five high expressor Triton WR 1339–treated female mice were fed by gastric tube 100 μCi of glycerol-[^{3}H]trioleate mixed with 100 μl of corn oil (25). Blood samples were drawn 30, 45, 60, and 90 min thereafter and the amounts of radioactivity in serum triglycerides were determined as described above.

In vivo apolipoprotein production. Triton-treated (500 mg/kg) control (n = 6) and high expressor transgenic mice (n = 6) were given 100 μCi [^{35}S]methionine intravenously and 60 min later were bled from the retroorbital plexus. We previously observed that labeled apolipoprotein secretion increased linearly past 60 min (unpublished observation; see reference 26). VLDL was isolated by ultracentrifugation (15) and the samples were examined by 4–15% gradient SDS-PAGE. After staining and destaining, the gels were immersed in EN^{3}HANCE solution (DuPont Co., Wilmington, DE) and fluorography of the dried gel was performed to visualize the radioactive proteins. The films were scanned with an UltroScan XL Laser Densitometer.

Tissue LPL activity. Two control and two high expressor transgenic mice with ad lib access to food were used for tissue LPL activity. 50–100 mg of epididymal fat was homogenized in 10 ml acetone using a Polytron homogenizer with a pt 10-11 probe at maximum speed for 2 min at 0°C. The homogenates were centrifuged at 10,000 rpm for 20 min at 4°C and the supernatants were discarded. The pellet was reextracted three times with 10 ml of ice-cold acetone and twice with diethyl ether. The dried powder was designated as acetone powder. Diaphragm and muscle tissues were homogenized in 0.025 M NH_3Cl buffer (pH 8.1) using the Polytron homogenizer. 0.1 ml of diaphragm or muscle homogenates containing 2–4 mg of fresh tissue or 0.1 ml of the acetone powder of adipose tissue containing 0.2–0.5 mg of the powder in 0.025 M NH_3Cl buffer (pH 8.1) were incubated with 0.1 ml of substrate containing 1 mg triolein (Intralipid), 200,000 dpm of [^{3}H]-glyceroltrioleate, and 16 μl of heat-inactivated fasted rat serum in 0.2 M Tris-HCl buffer (pH 8.1). After 45 min incubation at 37°C the reaction was stopped by addition of 3.25 ml of methanol-chloroform-heptane (1.4:1.21:1, vol/vol/vol). The extraction of fatty acids was performed according to the method of Nilsson-Ehle and Schotz (27). 1 mU of enzyme activity represents the release of 1 nmol of fatty acid per minute.

Purified LPL assay. To determine the ability of LPL to release fatty acids from VLDL triglycerides, LPL was purified from human milk using a method described previously (28). Protein concentration was measured using a commercial kit (Bio-Rad Laboratories). Control and high expressor VLDL were labeled in vivo by injecting a [^{3}H]palmitate albumin complex (19). The specific activity of triglycerides in control and transgenic VLDL was 2,843±272 and 1,000±303 dpm/μg, respectively. 0.03, 0.05, 0.075, 0.1, 0.2, 0.3, 0.5, 0.6, and 0.8 mM of VLDL triglycerides were incubated in the presence of 4% albumin and 10 mM Tris-HCl (pH 8.2) for 20 min with 65 ng of LPL. A blank sample without the addition of lipase was performed for each concentration and the difference was regarded as the hydrolyzed amount of VLDL triglycerides. Each reaction was stopped by adding 3.25 ml of a mixture of chloroform/methanol/n-heptane (1:1.28:1.37, vol/vol/vol) and 1 ml of 0.1 M K_2CO_3/H_3BO_3 (pH 10.5) (29). FFA were extracted by vortexing the mixture for 15 s and the phases were separated by centrifugation at 3,000 rpm at 4°C for 20 min. 1 ml of the upper phase was added to scintillation liquid (Ready Safe; Beckman Instruments, Inc., Fullerton, CA). The experiments were repeated eight times with three different VLDL preparations. Rates of lipolysis under these experimental conditions were linear for control and high expressor VLDL for at least 20 min (data not shown). The amount of substrate was not rate limiting since only 2–4% of VLDL triglycerides were hydrolyzed at each point. Apparent Michaelis constants (K_m) and maximal enzyme activities (V_{max}) for LPL assayed with control or transgenic VLDL were calculated from Lineweaver-Burk plots.

Tissue uptake of labeled VLDL. Rat hepatoma cells (McArdle cells) were incubated with either control or transgenic VLDL that were labeled in vivo with [^{3}H]glycerol (see above). All incubations were done with triglyceride-deficient medium. Plates were pretreated with 30 μM lovastatin for 18 h before the experiment to increase LDL receptor activity and the drug was maintained in the medium during the experiments. Incubation times of 2 and 6 h were used. For each experiment, plates were incubated with equal amounts of radioactivity in labeled control, low, or high expressor VLDL. Plates that received control or low expressor VLDL were also supplemented with unlabeled control or low expressor VLDL, respectively, to match the triglyceride concentration in plates with high expressor VLDL. The final triglyceride concentration in the experimental medium was ~ 0.4 mg/ml. Radioactivity in the medium and the cell-associated counts were determined after the incubations by scintillation counting using ReadySafe as the scintillation fluid. The cellular protein concentration in each plate was determined using a Lowry assay with BSA as the standard (30).

Free fatty acids. FFA levels were determined in plasma samples from animals with ad lib access to food in the morning (8 a.m. fed) and for evening samples (6 p.m. fasted) food was removed the same morning. 20 control, 10 low expressor, and 20 high expressor mice, both male and female, were bled from the retoorbital plexus at each time point. EDTA-containing capillaries were used and samples were kept on ice all the time. Plasma was separated and the amount of FFA was determined immediately after bleedings using a commercial kit according to instructions of the manufacturer (NEFA C; Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Preparation of hepatocytes for primary culture. Three control and three high expressor male mice were anesthetized with 5% sodium pentobarbital. The preparation of hepatocytes was performed using a method described previously by Sparks et al. (31) with minor modifications. Briefly, the portal vein was cannulated and the liver was perfused first with a calcium-free isotonic buffer at 37°C for 10–15 min and then with a second buffer containing 5 mM $CaCl_2$ and 0.075% (wt/vol) collagenase (Boehringer Mannheim Corp.) for 30 min. After the perfusion the cells were suspended in the calcium-free buffer. The cells were washed with PBS and M199 (Sigma Chemical Co., St. Louis, MO) and the dead cells were separated from the live ones by centrifugation in a solution containing 50% colloidal polyvinylpyrrolidone-coated silica (Percoll; Sigma Chemical Co.). The viability of the cells was determined by trypan blue staining. 500,000 live cells were plated on a 35-mm plate coated with poly-d-lysine. The culture medium was changed after a 4-h incubation. Experiments were performed the next day. As experimental medium, RPMI (Sigma Chemical Co.), was used supplemented either with 1 mM oleic acid complexed to 0.2 mM BSA or with 0.2 mM BSA alone. 70 μCi/ml of [^{35}S]methionine and 10 μCi/ml of [^{3}H]glycerol were added and the cells were incubated for 5 h at 37°C. After incubation the medium was removed and the radioactivity in the triglyceride fraction was measured by Dole's extraction method (32) and TLC on silica gel G plates (Analtech Inc.) using hexane-diethylether-acetic acid solution (83:16:1) as the solvent (19). The percentage of counts in triglyceride fractions was determined. More than 90% of counts were in the triglyceride fraction in oleic acid–treated cultures and ~ 80% in BSA-treated cultures. The amount of newly synthesized VLDL apo B was determined on a 4–15% gradient gel as described above (see above). The total amount of secreted proteins was measured by TCA precipitation (33). Cellular triglyceride mass was determined by incubating the washed cells with isopropanol for 18 h (34) and by

measuring the triglyceride concentration using a commercial kit (#701882; Boehringer Mannheim Corp.). The radioactivity in cellular triglycerides was determined using Dole's extraction method (32). Cellular protein concentration on each dish was determined by solubilizing the cells with 0.1 M NaOH and by performing a Lowry assay with BSA as standard (30). Only dishes with cellular protein concentration of > 500 μg were included in the study.

Statistical analysis. Results are given as mean±SD if not otherwise indicated. Any differences in triglyceride, cholesterol, and FFA concentrations, VLDL size distributions, various apolipoprotein and triglyceride amounts, triglyceride production, and tissue culture removal assays were calculated by analysis of variance (ANOVA) using multiple comparison techniques. Differences in the triglyceride production by hepatocytes and in lipase assays were determined using the *t* test for nonpaired samples.

For VLDL turnover studies, statistical analysis was done by two-way ANOVA with treatment (control, low, and high expressors) as a fixed effect and experimental day (four different days over a 4-month period) as a random effect. This allowed for the possibility of day-to-day variations in animal weights, triglyceride levels, or kinetics due to litter effects or other factors. Logarithms of triglycerides and fractional catabolic rate (FCR) were used in statistical analysis to achieve similar variances in the three groups. Statistical analysis was done with SAS from SAS Institute, Cary, NC.

Results

Serum lipid levels and the composition of lipoprotein particles. The histologic structure of the liver from control and high expressor mice was similar with no extra lipid accumulation in high expressor livers (data not shown). Triglyceride and cholesterol concentrations in total serum samples as well as in lipoprotein fractions are shown in Table I. The samples were drawn in the morning. For each lipid determination, plasma samples were pooled from 3–10 mice. Total serum triglyceride level was significantly increased in low and high expressor transgenic mice of both sexes. In high expressor mice, total serum cholesterol was also significantly increased. The increases were primarily in the VLDL fraction (Table I). Cholesterol and triglyceride levels in LDL were also slightly higher in transgenic animals than in controls (Table I). HDL cholesterol was significantly lower in transgenic male and female mice than in controls ($P < 0.01$ and $P < 0.04$, respectively).

The level of human apo CIII was found to be ~ 4.2 mg/dl in low expressor mice and ~ 30 mg/dl in high expressor mice. The concentrations in male and female mice did not differ from each other (data not shown). Human apo CIII was present in all lipoprotein fractions, but it was not detectable in the fraction of $d > 1.21$ (data not shown). In low expressor mice similar amounts of human apo CIII were found in the VLDL and HDL fractions, with < 5% in the LDL fraction. In high expressor mice approximately two-thirds of human apo CIII was in the VLDL fraction, one-third in the HDL fraction, and only a small amount in the LDL fraction.

The VLDL apolipoproteins separated by gradient SDS-PAGE were scanned (Fig. 1 *A*), and the amount of apo B (B48 and B100) loaded on each lane was calculated. Since there is only one apo B molecule per VLDL particle, the triglyceride content in a VLDL particle was expressed as VLDL triglycerides/VLDL apo B. VLDL particles obtained from high expressor mice contained ~ 50% more triglycerides than control

Table I. Serum Lipid and Lipoprotein Concentrations in Control, Low, and High Expressor Female and Male Mice

		Total		VLDL		LDL		HDL	
	n	tg	chol	tg	chol	tg	chol	tg	chol
Females									
Control	(6)	135±24	82±18	103±24	13±9	23±9	13±8	9±3	54±10
Low expressor	(4)	258±67	86±10	213±68	22±9	33±6	14±9	12±3	51±4
High expressor	(6)	957±158	145±21	905±164	79±14	37±9	26±12	12±11	39±11
P (ANOVA)		0.0001	0.0005	0.0001	0.0001	0.03	0.1	NS	0.04
Control vs. low expressor		0.0007	NS	0.001	NS	0.1	NS	NS	NS
Control vs. high expressor		0.0001	0.0006	0.0001	0.0001	0.01	0.06	NS	0.02
Low vs. high expressor		0.0001	0.003	0.0001	0.0004	NS	0.1	NS	0.09
Males									
Control	(7)	119±30	98±20	92±34	9±5	21±8	15±5	8±4	75±19
Low expressor	(3)	280±103	90±17	243±99	30±12	29±10	17±1	9±3	41±12
High expressor	(5)	1357±362	177±76	1294±359	104±49	56±33	34±16	9±2	39±18
P (ANOVA)		0.0001	0.03	0.0001	0.0005	0.04	0.01	NS	0.01
Control vs. low expressor		0.004	NS	0.008	NS	NS	NS	NS	0.03
Control vs. high expressor		0.0001	0.02	0.0001	0.0003	0.02	0.006	NS	0.007
Low vs. high expressor		0.0004	0.04	0.001	0.02	NS	0.06	NS	NS

Blood drawn 8 a.m. with the animals having ad lib access to food. Due to small losses during ultracentrifugations, lipid values in various lipoprotein fractions are corrected to match total lipid values (the values are expressed as mg/dl, mean±SD). *n* = number of pools each containing 3–10 mice.

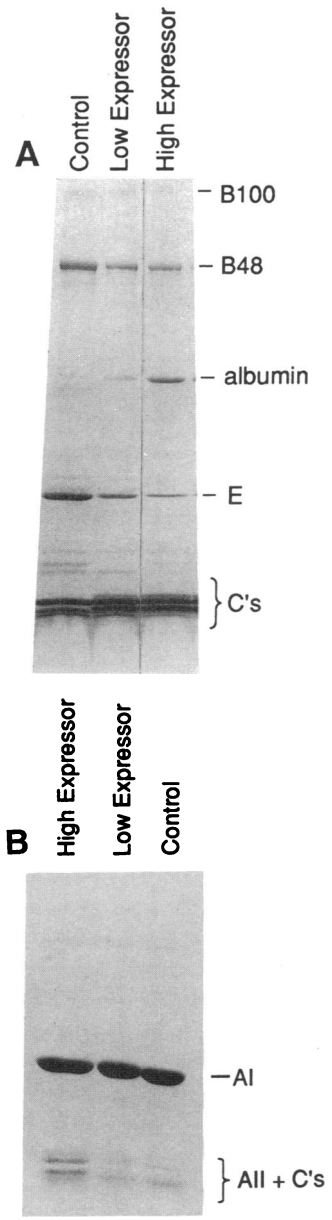

Figure 1. A 4–15% SDS-PAGE of VLDL (*A*) and HDL (*B*) apolipoproteins isolated from control and low and high expressor transgenic mice. Due to a small amount of apo AII in mouse HDL (35), it is not clearly resolved from the region of apo C's. 50 and 25 μg of VLDL and HDL apolipoproteins respectively, were loaded on each lane.

VLDL (Table II). The triglyceride content in low expressor VLDL was not different from control VLDL.

The amounts of various apolipoproteins in a VLDL particle were also expressed per VLDL apo B. The amount of apo E per VLDL particle was 65 and 23% in low and high expressor VLDL, respectively, compared with controls. The amount of all apo C's per VLDL particle was 75 and 180% higher in low and high expressor mice, respectively, than in controls (Fig. 1 *A* and Table II). In HDL particles from both control and transgenic mice, apo AI accounted for ~ 75% of the total protein and apo AII and all apo C's for the rest (Fig. 1 *B*). The amount of apo AI was the same despite the additional human apo CIII in transgenic HDL. No detectable apo E was observed in any HDL preparations.

Isoelectric focusing demonstrated that in VLDL from transgenic mice, mouse apo CII and apo CIII were decreased in both low and high expressor mice (Fig. 2 *A* and Table II). Of the human apo CIII isoforms, $CIII_0$ was the most prevalent in transgenic mice even though it is the least abundant in humans (Fig. 2 *A* and Table III). In low expressors, 57% of human apo CIII was in $CIII_0$ isoform and only 11% in apo $CIII_2$ isoform. In high expressors, only 42% was in apo $CIII_0$ isoform and 27% in apo $CIII_2$ isoform. In HDL from transgenic mice, human apo CIII's were present and mouse apo CII and CIII were at normal levels in both high and low expressors (Fig. 2 *B*).

Under electron microscopy VLDL particles measured 51.0±13.4 nm (52.3±15.5; 49.7±11.1), 55.2±13.6 nm (57.3±15.2; 53.4±11.9), and 58.2±17.8 nm (57.4±19.4; 59.1±16.1) (mean±SD; numbers in parentheses are for individual mice) in control, low, and high expressor mice, respectively, from blood samples drawn at 8 a.m. ($P < 0.0001$). The size distribution was different in the three lines, with more of the larger VLDL particles in the transgenic animals (Fig. 3).

Overall, VLDL particles from high expressor mice were more triglyceride-rich than control VLDL. Also, under electron microscopy VLDL particles from low and high expressor animals seemed slightly larger than control VLDL. Since the triglyceride content of the particles was increased only 50%, the number of VLDL particles in the circulation was elevated about 600%. The total amount of apo CIII increased on both low and high expressor VLDL particles, but the amounts of mouse apo E, CII, and CIII decreased on VLDL particles in both transgenic mouse lines.

In vivo mechanisms. Several in vivo assays were used to examine whether VLDL production or removal was affected in these HuCIIITg mice.

To study the removal of VLDL particles, control, low, and high expressor mice were injected with 200,000 dpm of in vivo radiolabeled VLDL from control or high expressor mice. Due to rapid exchange of surface components (35), no differences in the disappearance of radiolabeled VLDL were observed

Table II. The Amount of Various Apolipoproteins and Triglyceride per VLDL apo B in high and low expressor mice*

	apoE	apoCs	apoCII	mouse apoCIII	total apoCIII	triglyceride
Control	100	100	100	100	100	100
Low expressor	65±20	175±14	52±15	76±12	196±11	107±26
High expressor	23±5	280±36	49±11	58±5	252±25	157±17
P (ANOVA)	0.002	0.0002	0.004	0.0003	<0.0001	0.02
Control vs. low expressor	0.03	0.02	0.02	0.004	<0.0001	NS
Control vs. high expressor	0.002	0.0006	0.005	0.0008	<0.0001	0.02
Low vs. high expressor	0.05	0.005	NS	0.03	0.004	0.03

For each VLDL, four separate VLDL preparations were used. The amount of proteins in each lane was determined by scanning the Coomassie blue-stained bands. (Percentage of the normal amount, mean±SD). * apo B48 and apo B100 combined.

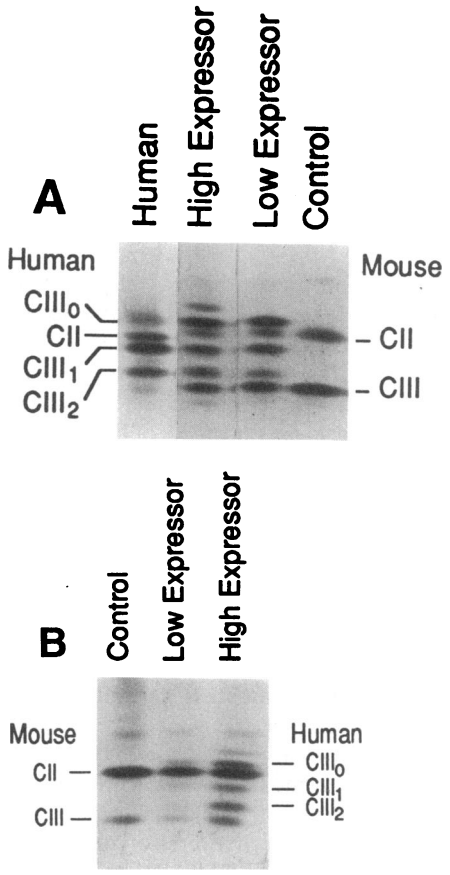

Figure 2. Isoelectric focusing of apo C's in VLDL (*A*) and HDL particles (*B*) isolated from control and low and high expressor mice. Delipidation of the lipoproteins was performed with acetone-ethanol (1:1), and 50 and 25 μg of VLDL and HDL apolipoproteins, respectively, were loaded on each lane. A gel with pH range of 4–6.5 was used.

whether control or high expressor VLDL was used. In addition, no differences were found whether the experiment was performed with male or female mice. VLDL particles containing triglycerides labeled either with radioactive glycerol or fatty acid had the same removal rates (data not shown). The data analysis for each control mouse and all but one low expressor required a two-pool kinetic model, while 8 of 12 high expressors were adequately fitted by a single-pool model. Typical curves from each of the three groups are shown in Fig. 4. There was significant day-to-day variation in the recovery of the injected tracer, but there was no difference in the recovery among the three experimental groups.

The data on weight, triglyceride levels, FCR, and production rate (PR) are shown in Table IV. The analysis showed significant day-to-day variation as well as significant interaction for all four variables. Using the interaction term as the error term, the weight of the mice did not differ from each other in the three groups. Triglycerides were significantly elevated in both low and high expressor mice ($P < 0.02$ and 0.0001, respectively). VLDL FCR was decreased from the control level of 7.67 pools/h to 2.58 pools/h in low expressor mice ($P = 0.07$) and 0.77 pools/h in high expressor mice ($P = 0.002$). The difference in FCR between low and high expressors was also significant ($P = 0.04$). PR was not significantly different in control compared with transgenic mice. With triglycerides elevated and FCR decreased in transgenics, a possible association of VLDL triglycerides and FCR was explored by computing correlations within each of the three groups. In the high expressors, log (triglycerides) had an inverse correlation of −0.58 ($P < 0.05$) with log(FCR). The inverse correlation was weaker and not significant in the low expressors ($r = -0.4$, $P = 0.25$) and absent in controls. No correlation was seen between triglyceride levels and PR.

In vivo triglyceride production was determined after blocking the lipase system with Triton WR 1339. The extent of inhibiting the lipase system was confirmed by giving Triton-treated mice in vivo labeled VLDL intravenously and by following the disappearance of radioactivity for 90 min in control and high expressor animals with serum triglycerides ranging from 70 to 120 and 510 to 870 mg/dl, respectively. In both types of mice > 70% of the radioactivity was still present in plasma 90 min after injection (data not shown) as compared with 13 and 45% found in untreated control and high expressor mice, respectively. In triglyceride production studies, mice were given ^{3}H-labeled glycerol 15 min after Triton injection. High expressor mice with serum triglyceride levels of 540–1,484 mg/dl produced twice as many labeled triglycerides as control mice with serum triglyceride levels of 72–270 mg/dl ($P < 0.005$; Fig. 5). Low expressor mice with serum triglycerides of 280–520 mg/dl produced slightly more than controls, but the difference was not statistically significant (Fig. 5). The difference between HuCIIITg mice and controls was probably not due to different precursor pools, since the specific activity of triglycerides in the liver 60 min after [^{3}H]glycerol injection was 106,614±6,514 dpm/mg triglycerides in high expressor mice ($n = 5$) and 103,308±9,421 dpm/mg triglycerides in controls ($n = 6$) ($P > 0.8$, mean±SE). In Triton-treated control and high expressor mice fed radiolabeled triglycerides through a feeding tube, there were no differences in the production of radiolabeled plasma triglycerides in the two mouse groups (data not shown), suggesting that the intestine is not the source of increased triglyceride production in transgenic mice.

To study in vivo apolipoprotein production, control and high expressor mice were given Triton WR 1339 intravenously (500 mg/kg) and 15 min thereafter [^{35}S]methionine intravenously. Blood samples were collected 60 min after the methionine injection. The lipoprotein fraction with a density of < 1.006 was isolated by ultracentrifugation. The samples were concentrated using commercial Centricon 30 tubes (Amicon, Beverly, MA) and labeled apolipoproteins separated on a 4–15% SDS gel. Radioactivity in each protein was determined by scanning the fluorographs of the dried SDS gels. After scan-

Table III. The Distribution Expressed as Percentage of Various Human apo CIII Isoforms in VLDL Obtained from Low and High Expressor Transgenic Mice and from a Normal Human VLDL Sample

VLDL	n	$CIII_0$	$CIII_1$	$CIII_2$
Human	(2)	13.5±0.7	55.0±1.4	31.5±0.7
Low expressor mice	(5)	57.5±2.1	31.0±2.9	11.8±1.8
High expressor mice	(5)	42.4±1.4	32.2±1.8	25.0±1.2

(Mean±SD, n = number of different VLDL preparations scanned).

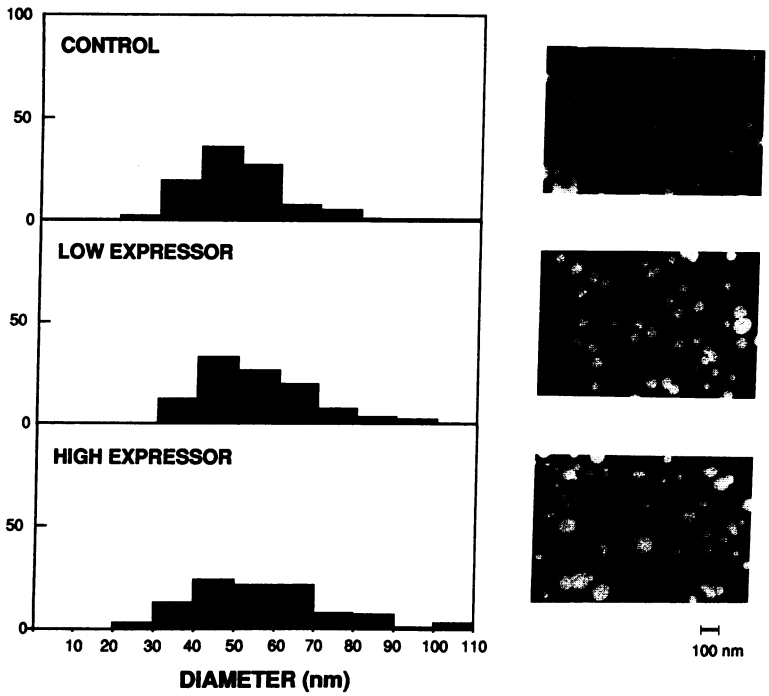

Figure 3. The size distribution of VLDL particles obtained from high and low expressor transgenic mice and from controls. Under electron microscopy, > 100 particles were measured in two control, two low expressor, and two high expressor mice. Since the distributions in the two animals in the same group did not differ significantly from each other, the combined size distribution in each group is shown.

ning the films no significant differences were observed in the amount of apo E and apo B synthesized by the two groups (Table V). However, the production of apo C's was increased more than twofold in the high expressor transgenic mice (Table V).

In summary, the in vivo studies showed that the FCR of VLDL particles was significantly reduced in both transgenic mouse lines. Triglyceride production by liver was also found to be increased in these mice.

Mechanisms for decreased FCR. LPL activity and tissue uptake of VLDL particles were measured to determine the mechanism for the decrease observed in vivo in VLDL FCR.

Tissue LPL activity was measured in muscle and in adipose tissues and no differences were found in control versus high expressor transgenic mice (Table VI). When purified LPL was added to increasing amounts of labeled control or high expressor VLDL, there were no significant differences in the amount of fatty acids released from the two types of VLDL particles (Fig. 6). The apparent K_m was calculated to be 1.14 and 1.39 mM for control and transgenic VLDL triglycerides, respectively, and the apparent V_{max} was 0.550 mmol fatty acids released/h per mg LPL for control VLDL triglycerides as compared with 0.528 for transgenic VLDL triglycerides. The two sets of values did not differ significantly from each other.

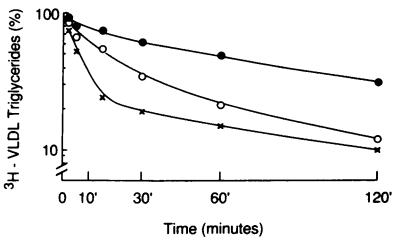

Figure 4. The removal of labeled VLDL in a typical control (×), low (○), and high (●) expressor mouse. Mice were injected with 200,000 dpm of in vivo radiolabeled VLDL. 60 μl of blood was drawn at each time point and the radioactivity of the serum sample was measured.

Tissue uptake was investigated in rat hepatoma cell cultures. In preliminary experiments the same amount of VLDL was removed from the culture medium whether VLDL was labeled with ^{125}I or [^{3}H]glycerol. In addition, the uptake of labeled VLDL particles was enhanced if lovastatin was added to the culture medium. In hepatoma cell culture studies, control VLDL was removed faster from the culture medium than VLDL from low expressor mice, which was removed faster than VLDL from high expressor mice (Table VII). Since the amount of triglycerides added to the culture medium was kept constant and high expressor mice had a 50% increase in VLDL triglyceride content, the actual number of high expressor VLDL particles taken up was even less compared with control VLDL. Thus the difference in uptake of transgenic and control VLDL is actually greater than it appears in Table VII.

These data suggest that the decreased VLDL FCR in HuCIIITg mice is primarily due to decreased cellular uptake of apo CIII-rich VLDL particles.

Mechanism for increase in triglyceride production rate. The levels of serum FFA and triglyceride secretion by hepatocytes were studied to examine the possibility of increased triglyceride production in the transgenic mice as suggested by experiments with Triton-treated animals.

FFA concentrations were found to be elevated in high expressor transgenic mice in both fed and fasting plasma samples (Table VIII). In low expressor mice, the FFA level was elevated only in fasting samples, while fed samples resembled the values found in control mice (Table VIII).

Triglyceride synthesis and secretion were studied in primary hepatocyte cultures obtained from control and high expressor mice incubated with [^{3}H]glycerol. In the basal situation (medium supplemented with BSA), control and HuCIIITg hepatocytes had the same cellular triglyceride mass and radioactivity and medium triglyceride radioactivity (Table IX). 1 mM oleic acid stimulated intracellular triglyceride mass and radioactivity in control and HuCIIITg hepatocytes. The accumulation

Table IV. VLDL Triglycerides Fractional Catabolic Rates and Production Rates for Control, Low, and High Expressor Mice

	n	Weight	Triglycerides	FCR	PR
		g	*mg/dl*	*pools/h*	*mg/h per g*
Control	(17)	28.7±3.0	152±46	7.67±4.99	0.392±0.301
Low expressor	(11)	30.2±6.5	279±140	2.58±1.45	0.220±0.144
High expressor	(12)	27.8±2.4	891±340	0.77±0.35	0.208±0.089
P (ANOVA)		NS	<0.0001	0.007	NS
Control vs. low expressor		NS	0.02	0.07	NS
Control vs. high expressor		NS	<0.0001	0.002	NS
Low vs. high expressor		NS	0.0006	0.04	NS

(*n* = number of animals, mean±SD). Due to effect of day on the various parameters, the statistics were calculated using the mean values per day per group (four mean values for each parameter in each group of mice).

of radiolabeled triglycerides in the culture medium in the presence of oleic acid was significantly greater from the transgenic compared with the control hepatocytes ($P < 0.01$, Table IX). The amount of newly synthesized VLDL apo B secreted by control or transgenic hepatocytes was, however, not different in the basal situation or in the presence of oleic acid (data not shown). Protein secretion from cells incubated with [^{35}S]-methionine was estimated as TCA-precipitable ^{35}S-radioactivity. This was the same for control and transgenic hepatocytes and was not affected by oleic acid treatment.

Increased FFA levels in vivo as well as elevated triglyceride, but not apo B secretion in transgenic hepatocytes are compatible with in vivo findings showing an increase in VLDL triglyceride but not apo B production. These studies suggest increased synthesis and secretion of VLDL triglycerides rather than decreased reuptake of the newly synthesized VLDL particles in these HuCIIITg mice.

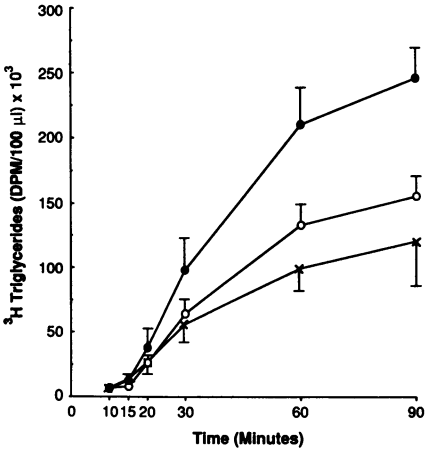

Figure 5. Triglyceride production in Triton WR 1339–treated control ($n = 19$) (×), low ($n = 10$) (○), and high ($n = 14$) (●) expressor transgenic mice after injection of [^{3}H]glycerol. Mice were given Triton WR 1339 intravenously and 15 min later 100 µCi of [^{3}H]glycerol. At each time point 60 µl of blood was drawn. The radioactivity of the serum sample was measured and TLC was performed to calculate the triglyceride-associated count. The triglyceride-associated counts were calculated for 100 µl of serum at each time point (mean±SE).

Discussion

The HuCIIITg mouse is the first animal model of primary hypertriglyceridemia. HuCIIITg mice demonstrate the importance of apo CIII in triglyceride metabolism and offer an opportunity to study the mechanism of hypertriglyceridemia. In HuCIIITg mice, hypertriglyceridemia was mainly due to an increased number of VLDL particles in the circulation. In addition, transgenic VLDL was larger and more triglyceride enriched than control VLDL and had an altered apolipoprotein content with increased total apo CIII and decreased apo E and apo CII.

Several types of studies of VLDL metabolism in vivo were undertaken to determine whether the increase in VLDL was due to increased synthesis, decreased catabolism, or both. The most striking results were obtained by kinetic modeling of radiolabeled VLDL-turnover studies. High expressor mice with a sixfold increase in triglycerides had one-tenth the normal VLDL triglyceride FCR, whereas low expressor mice with a twofold increase in triglycerides had one-third the normal VLDL triglyceride FCR. Thus, by this technique the hypertriglyceridemia in HuCIIITg mice is adequately explained by diminished VLDL catabolism.

One possible mechanism to explain diminished VLDL catabolism in HuCIIITg mice is that excess apo CIII inhibits VLDL triglyceride hydrolysis by LPL. Transgenic animals were found to have normal levels of LPL activity in muscle and

Table V. The Amount of Different Apolipoproteins Synthesized in Triton-treated Control and High Expressor Mice 60 Min after They Received 100 µCi ^{35}S-Methionine Intravenously

	n	apoB*	apoE	apoCs
Control	(6)	100	100	100
High expressor	(6)	96±29	122±62	234±70
P		NS	NS	0.003

In each experiment the mean of each apolipoprotein value in controls was calculated and each high expressor value was expressed relative to that (*n* = number of animals, percentage of mean control value, mean±SD). * apo B 48 and 100 combined.

Table VI. Tissue Lipoprotein Lipase Activity in Control and High Expressor Mice

Organ	Control	High expressor
Diaphragm	546 (539, 552)	541 (528, 554)
Gastrocnemius	158 (174, 142)	167 (175, 159)
Epididymal fat pad*	15033 (17164, 12902)	17650 (14638, 20562)

Two mice were examined in both groups. (Mean of the two values, the individual values as shown, data expressed as nanomoles of fatty acids released per minute per gram fresh tissue.)
* Nanomoles of fatty acids released per minute per gram of acetone powder.

adipose tissue. In addition, purified LPL hydrolyzed triglycerides in control and transgenic VLDL with equal apparent K_m and V_{max}. These studies suggest that apo CIII in the HuCIIITg mice does not cause reduced VLDL FCR by suppressing the amount of extractable LPL in tissues or by making HuCIIITg VLDL a bad substrate for LPL. Previous studies had shown that relatively large amounts of exogenous apo CIII inhibited LPL hydrolysis of triglycerides in artificial VLDL (9–11). A molar ratio of apo CIII to apo CII of 20:1 was required to inhibit LPL by 50% (11). The molar ratio of apo CIII to apo CII in high expressor mice is only 5:1. Excess apo CIII in transgenic VLDL could also inhibit LPL by displacing its cofactor, apo CII. In fact, apo CII is diminished by 50%, but this is probably not meaningful, since obligate heterozygotes for apo CII deficiency have normal triglyceride levels (36). Although we could not show that HuCIIITg VLDL is a bad substrate for LPL in vitro, it is still possible that diminished hydrolysis of HuCIIITg

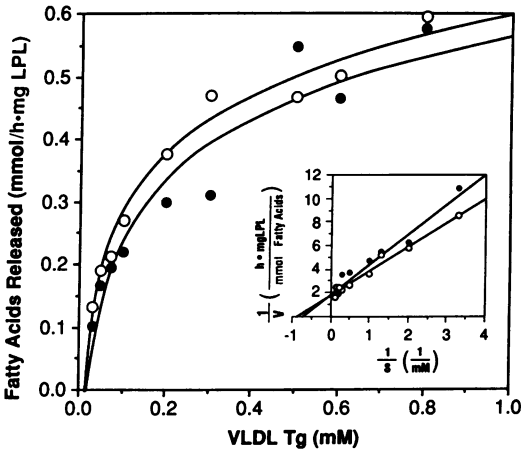

Figure 6. The amount of FFA released by LPL from ^{3}H-VLDL triglycerides obtained from control (○) or high expressor transgenic (●) mice. Purified LPL was incubated with different amounts of in vivo labeled VLDL for 20 min at 37°C. The reaction was stopped by adding 3.25 ml of chloroform/methanol/*n*-heptane (1:1.28/1.37, vol/vol/vol) mixture and 1 ml of 0.1 M K_2CO_3/H_3PO_4 (pH 10.5). FFA were extracted by vortexing the mixture and the phases were separated by centrifugation at 3,000 rpm at +4°C for 20 min. The radioactivity of the upper phase was measured using Ready Safe scintillation liquid. Inset shows Lineweaver-Burk plots that were contracted using data in the large figure and were used to calculate apparent K_m and V_{max} for both VLDLs.

Table VII. The Amount of VLDL Triglycerides Taken Up by Rat Hepatoma Cells Incubated with Control, Low, or High Expressor VLDL in the Presence of 30 μM Lovastatin

	2 h	6 h
Control	16.2±1.8	47.3±5.6
(*n*)	(4)	(5)
Low expressor	6.3±2.8	16.6±1.8
(*n*)	(4)	(5)
High expressor	5.7±1.6	9.6±7.6
(*n*)	(4)	(5)
P (ANOVA)	0.0001	0.0001
Control vs. low expressor	0.0006	0.0001
Control vs. high expressor	0.0004	0.0001
Low vs. high expressor	NS	0.08

(*n* = number of plates, units are micrograms triglycerides per milligram cellular protein±SD).

VLDL occurs in vivo. The fact that HuCIIITg VLDL has a prolonged residence time yet remains larger than control VLDL suggests diminished triglyceride hydrolysis in vivo. One possible mechanism could be apo CIII inactivation of endothelial surface-bound LPL by blocking its interaction with VLDL or the LPL cofactor apo CII.

Another mechanism that could explain the decreased VLDL triglyceride FCR in HuCIIITg mice is diminished tissue removal of transgenic VLDL particles. Transgenic VLDL were shown to have diminished uptake by rat hepatoma cells in tissue culture. The difference between the uptake of control and transgenic VLDL was enhanced by pretreating the cells with lovastatin, suggesting an LDL receptor-mediated process. Previously it has been shown that rat (37, 38) and human hypertriglyceridemic (39, 40) VLDL uptake can occur through recognition of apo E on the VLDL by LDL receptors. This process was greatly increased by pretreating the VLDL with LPL (37) and it was suggested that the lipase allowed a conformational change in apo E to take place which in turn allowed receptor recognition. The diminished apo E in transgenic VLDL may prevent recognition by tissue LDL receptors, or the presence of excess apo CIII on the particles could prevent the conformational change in apo E required for efficient removal. One or both of these mechanisms may be operative to explain the diminished VLDL triglyceride FCR in HuCIIITg mice. Similar findings have also been presented previously by Windler and co-workers (12, 41, 42). They showed that triglyceride-rich particles enriched with apo C's, including apo CIII, were cleared to a smaller extent than control particles by perfused rat livers. They concluded from these studies that the inhibition by apo CIII might be independent of the amount of apo E on the particles. However, they could not rule out the possibility that apo CIII would alter the conformation of apo E and inhibit binding in that way.

It was recently shown that βVLDL recognition by LDL receptor related protein (LRP) is mediated by apo E and that apo CI, but not apo CIII, displaces apo E from βVLDL and interferes with binding (43, 44). Our experiments in vivo and those of Sehayek and Eisenberg (45) in tissue culture strongly suggest that normal VLDL is cleared by LDL receptors and apo

Table VIII. The Concentration of Free Fatty Acids (mM) in Fed (8 a.m.) and Fasted (6 p.m.) Conditions

	All		Female		Male	
	8 a.m.	6 p.m.	8 a.m.	6 p.m.	8 a.m.	6 p.m.
Control	0.383±0.146	0.604±0.178	0.310±0.105	0.600±0.137	0.456±0.150	0.607±0.219
(*n*)	(20)	(20)	(10)	(10)	(10)	(10)
Low expressor	0.387±0.115	0.803±0.159	0.402±0.120	0.810±0.151	0.372±0.121	0.796±0.184
(*n*)	(10)	(10)	(5)	(5)	(5)	(5)
High expressor	0.648±0.249	0.848±0.202	0.524±0.213	0.870±0.125	0.766±0.240	0.825±0.263
(*n*)	(20)	(20)	(10)	(10)	(10)	(10)
P (ANOVA)	0.0001	0.0004	0.02	0.0006	0.0008	0.11
Control vs. low expressor	NS	0.009	NS	0.01	NS	0.16
Control vs. high expressor	0.0001	0.0002	0.008	0.0003	0.002	0.05
Low vs. high expressor	0.002	NS	NS	NS	0.002	NS

(*n* = number of animals, mean±SD).

CIII does have a modulatory role. Sehayek and Eisenberg also suggested that apo CI can play a role in normal VLDL clearance (45) and Simonet and co-workers (46) have recently shown that apo CI transgenic mice display a mild hypertriglyceridemia. In the aggregate, these studies suggest that the clearance of βVLDL by LRP is mediated by recognition of apo E with apo CI as the principal modulator, and the clearance of normal VLDL by LDL receptors is also mediated by recognition of apo E, but now with apo CIII as the principal modulator.

It is of interest that inhibition of VLDL removal by apo CIII excess does not result in the accumulation of cholesterol ester–rich remnant particles as does the inhibition of VLDL removal caused by genetically defective apo E. Our previously published plasma lipoprotein pattern by fast protein liquid chromatography (14) and the electron microscopic and chemical data presented here suggest that in HuCIIITg mice normal or slightly triglyceride-enriched VLDL particles accumulate. Thus, our studies suggest that in vivo a significant amount of VLDL is cleared before it is transformed to a remnant particle and that apo CIII is regulating this step rather than a later step in VLDL catabolism which would result in the accumulation of remnants.

Although the radiolabeled VLDL turnover studies suggested that the increased triglycerides in HuCIIITg mice was due to diminished VLDL FCR with no significant differences in triglyceride production rate, other types of studies indicated oversynthesis of VLDL triglycerides. In animals treated with Triton to block VLDL clearance, we observed an increased rate of incorporation of [^{3}H]glycerol into VLDL triglycerides in transgenic mice compared with controls when the label was given intravenously, but not when it was given intragastrically. In Triton-treated animals, there was no increased rate of incorporation of intravenous [^{35}S]methionine into VLDL apo B in transgenic mice compared with controls. These triglyceride production results are somewhat contradictory. This could be because we were forced to fit the VLDL turnover data in the high expressor mice with a single pool model, perhaps due to an inability to get early enough time points in an animal as small as the mouse. A single-pool model precludes the accurate detection of a rapidly metabolized pool and could underestimate the actual production rate. In control animals the VLDL turn-

Table IX. Protein and Triglyceride Levels in Primary Hepatocytes and in their Culture Medium Treated with BSA or Complexes of BSA-Oleic Acid (OA)

		Intracellular			Medium	
	n	Protein	Triglycerides		Triglycerides	TCA precipitable
		μg/dish	*mg/mg cell protein*	*dpm/mg cell protein**	*dpm/mg cell protein**	*dpm/cell protein*
Control-BSA	(9)	734±41	0.15±0.01	194,400±8,736‡	15,110±1,060§	21,262±996
Control-OA	(8)	730±49	0.19±0.02	726,778±120,757‡	22,625±1,375¶§	19,144±1,244
Transgenic-BSA	(10)	672±23	0.14±0.01§	220,667±17,677‡	21,200±3,325‖	22,940±891
Transgenic-OA	(9)	661±25	0.18±0.01§	665,750±109,218‡	41,111±7,600‖¶	22,684±1,225

(*n* = number of plates, mean±SD). * Due to differences in SD, statistical analysis done after logarithmic transformations. ¶ $P < 0.01$ control hepatocytes compared with transgenic hepatocytes. § $P < 0.05$, ‖ $P < 0.001$, ‡ $P < 0.0001$ BSA-treated compared with oleic acid–treated hepatocytes obtained from the same animals.

over data could be fit with a two-pool model and more accurately measure production rate. In the Triton-treated animal method, production rate in both transgenics and controls is measured more directly. In support of the in vivo studies with Triton-treated animals, in the presence of oleic acid primary hepatocytes from HuCIIITg mice secreted into the medium twice the amount of triglycerides, but not apo B, as hepatocytes from control mice. In addition, plasma FFA levels were higher in vivo in HuCIIITg mice than in controls. Others have shown that FFA levels drive hepatic VLDL triglyceride production (47, 48). All of these experiments suggest that the HuCIIITg mice have an increased production of liver-derived VLDL triglycerides, but not the total number of VLDL particles. This is compatible with the larger VLDL size under electron microscopy and the increased VLDL triglyceride/apo B ratio observed in transgenic VLDL. It is unlikely that the observed increased triglyceride appearance would be due to decreased reuptake of newly synthesized VLDL particles, since the appearance of newly synthesized apo B is unaltered.

In summary, the mechanism of hypertriglyceridemia in HuCIIITg mice appears to be primarily due to a failure to promptly clear VLDL from the circulation. This appears to be due to altered VLDL apolipoprotein composition, which affects tissue recognition by LDL receptors. The greatly enlarged pool of circulating triglyceride-rich VLDL particles could cause increased plasma FFA concentration by saturating peripheral removal mechanisms. Increased plasma FFA would in turn come back to the liver and cause increased VLDL triglyceride production. The altered lipoprotein phenotype and metabolic studies in HuCIIITg mice suggest that they are a model for primary familial hypertriglyceridemia. In these individuals, triglyceride-enriched VLDL (49, 50) accumulate in plasma and turnover studies show reduced removal of VLDL triglycerides (51, 52). This contrasts with turnover studies in hypertriglyceridemic obese individuals, which indicate a major increase in VLDL triglyceride production (51, 53, 54), and studies in patients with familial combined hyperlipidemia which indicate increased VLDL apo B production rate (51, 55–57) and increased numbers of normal-sized VLDL particles (51, 52). Thus the HuCIIITg mouse mainly resembles primary familial hypertriglyceridemia and this study provides a possible mechanism for this human condition.

Acknowledgments

We want to thank Dr. E. Sphilas for the electron microscopy, Dr. C. Moberg for revising this manuscript, and Ms. K. Tsang for expert technical assistance. We are also very grateful to Dr. T. M. Forte for helpful discussions and suggestions.

This work was supported by grants from the National Institutes of Health (HL-32435, HL-33714, HL-22263, and PHS TW04366), in addition to funds from the Finnish Academy of Sciences, the Finnish Cultural Foundation, and the Helsinki Heart Study Foundation (K. Aalto-Setälä), and the W. W. Smith Charitable Trust (E. A. Fisher and X. Chen).

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