Resistance of chylomicron and VLDL remnants to post-heparin lipolysis in ApoE-deficient mice: the role of apoE in lipoprotein lipase-mediated lipolysis in vivo and in vitro

Eva Zsigmond, Yasu Fuke, Lan Li, Kunihisa Kobayashi, and Lawrence Chan[1]

Departments of Cell Biology and Medicine, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030

Abstract **The interaction of lipoprotein lipase (LPL) with triglyceride-rich lipoproteins is governed by a number of factors, such as apolipoprotein (apo) C-II. The role of apoE in lipolysis is controversial. We made the unexpected observation that apoE-deficient mice were resistant to heparin-induced lipolysis; this study aims at examining the underlying mechanism for this observation. Compared to wild-type mice, apoE-deficient mice had significantly higher very low density lipoprotein (VLDL) and chylomicron remnant (VLDL/CMR) concentrations and moderately lower lipase activity (15.5 ± 1.3 mU/ml vs. 22.9 ± 2.5 mU/ml). Unlike in wild-type mice where the injection of heparin reduced total plasma triglycerides by 50% and VLDL/CMR triglycerides by over 95%, the injection of heparin into apoE-deficient mice did not significantly affect plasma lipids. Similarly, in vitro, purified human LPL (hLPL) almost completely hydrolyzed VLDL/CMR isolated from wild-type mice, but had no effect on VLDL/CMR from apoE-deficient mice. However, when the amount of apoE-deficient VLDL/CMR was reduced to an equivalent level as in wild-type mice, LPL hydrolyzed 94% of VLDL/CMR triglycerides. In order to increase the ratio of LPL to VLDL/CMR in vivo, we injected an adenovirus containing the human LPL cDNA into apoE-deficient mice, which produced marked liver-specific overexpression of LPL and significant reduction of VLDL/CMR (93%) and total plasma triglyceride concentrations (87%). Thus, apoE is not required for LPL activity in vivo or in vitro. Under certain pathological conditions, such as severe hyperlipidemia, the LPL pathway may be saturated and efficient lipolysis can proceed only if the ratio of substrate particles to LPL is adjusted to a more normal range.—**Zsigmond, E., Y. Fuke, L. Li, K. Kobayashi, and L. Chan. **Resistance of chylomicron and VLDL remnants to post-heparin lipolysis in apoE-deficient mice: the role of apoE in lipoprotein lipase-mediated lipolysis in vivo and in vitro. *J. Lipid Res.* 1998.** 39: **1852–1861.**

Supplementary key words hyperlipidemia • VLDL • chylomicron • catabolism • gene therapy

The clearance of plasma lipoprotein particles requires specific interactions with enzymes, receptors and ligands. Lipoprotein lipase (LPL), a lipolytic enzyme anchored to the endothelial surface of blood vessels by heparan sulfate proteoglycans, is the rate-limiting factor in the clearance of the triglyceride-rich lipoproteins, very low density lipoproteins (VLDL) and chylomicrons. There is renewed interest in studying lipolysis because postprandial lipemia is emerging as a potentially important risk factor for the development of atherosclerosis (1).

The mechanism of lipolysis is not fully understood; geometric, as well as compositional, factors affect LPL's interactions with its lipoprotein substrates and hence efficiency of lipoprotein catabolism. Lipolysis rates depend on the amounts of LPL, proper attachment of the enzyme to the endothelial surface of blood vessels, rate of blood flow, particle size, and apolipoprotein composition of the lipoprotein substrates (2). Under normal physiological conditions the limiting factor in the clearance of lipoproteins is probably not the amount of LPL, but rather the ability of lipoproteins to come into contact with the enzyme (3). The observation that heparin-induced release of LPL into the circulation increases the rate of enzyme–substrate interaction, resulting in rapid clearance of triglyceride-rich lipoproteins from the circulation, supports this view. The importance of lipoprotein size in influencing lipolysis rates is demonstrated by the fact that large postprandial chylomicrons have a greater probability to come into contact with LPL, and therefore have significantly faster clearance rates ($T_{1/2} \sim 10$ min) than smaller endogenous VLDL particles ($T_{1/2} \sim 4$ h) (2).

Efficiency of lipase action also depends on the apolipoprotein composition. Apolipoprotein (apo)C-II is an activator of LPL (4) and a deficiency of this apolipoprotein markedly impairs LPL action and is associated with severe

Abbreviations: LPL, lipoprotein lipase; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; CMR, chylomicron remnant; Ad.hLPL, adenovirus construct containing the human LPL gene; Ad.Luc, adenovirus construct containing the luciferase gene.

[1]To whom correspondence should be addressed.

hypertriglyceridemia (5). ApoC-III has the opposite effect by acting as an LPL inhibitor (6, 7). The role of apoE in the lipolysis reaction is controversial, but it has been suggested that apoE facilitates LPL action by anchoring lipoproteins to the endothelial surface adjacent to the enzyme (reviewed in ref. 2). ApoE, a glycoprotein with strong heparin-binding properties, may facilitate the interaction of lipoproteins with membrane-bound heparan sulfate proteoglycans (8, 9). Mutations in apoE that alter their ability to bind to heparan sulfate proteoglycans have been associated with dyslipoproteinemia (10), but other studies showed that apoE-deficient patients do not always have elevated triglycerides (11, 12). Numerous in vitro studies using artificial subtrates have examined the role of apoE in lipolysis, but the results are inconclusive, as there are reports that showed activation (13, 14), inhibition (15), and no effect (16) of apoE on lipolysis. It has been suggested that apoE stimulates lipolysis when it is associated with triglyceride-rich lipoproteins, but it is inhibitory to lipolysis when it is associated with triglyceride-poor lipoproteins, or when it is not associated with lipoprotein particles (13).

The apoE-deficient mouse model is widely used to study the role of apoE in lipoprotein metabolism, as well as the long-term metabolic effects of hyperlipidemia (17, 18). These animals have significantly elevated plasma VLDL and chylomicron remnant levels and their severe hypercholesterolemia is associated with accelerated atherosclerosis (18, 19). Although LPL is responsible for the hydrolysis of VLDL and chylomicrons, lipolysis has not been systematically studied in apoE-deficient mice. It was the aim of our study to examine lipase action in mice devoid of functional apoE. We were surprised to find that the hyperlipidemia in apoE-deficient mice was resistant to heparin-induced lipolysis. We have therefore investigated the potential role of apoE in LPL-mediated lipolysis in vitro and in vivo and the underlying mechanism for the apparent resistance of these mice to heparin.

MATERIALS AND METHODS

Animals

Male, 12–16 weeks old, wild-type (FVB) and apoE-deficient (129ola × C57BL/6) mice were fed ad libidum a standard mouse chow diet (Purina Chow 5001, Ralston Purina Co. St. Louis, MO) with free access to water. Animals were maintained at 12 h light and dark cycles. All procedures involving animals were performed according to the guidelines of the Animal Review Committee of Baylor College of Medicine. After a 4–6-h fast, anesthetized mice were bled from the tail vein into iced, EDTA-treated microcentrifuge tubes. For experiments that required post-heparin plasma, mice were injected intravenously with 100 U/kg body weight of heparin 5 min prior to bleeding. Plasma samples were stored at 4°C for lipid and lipoprotein analyses, or snap-frozen in liquid nitrogen and stored at −80°C when used for lipase activity assays.

Measurement of LPL activity

Plasma samples (20 μl) from untreated or heparin-injected mice were assayed for lipase activity according to a previously described method (20). Because 1 m NaCl inhibits LPL but not hepatic lipase, LPL activity was calculated by subtracting lipase activity in the presence of 1 m NaCl from total lipase activity, measured in the absence of 1 m NaCl. LPL activity was expressed as milliunits/ml (1 milliunit = 1 nmol of free fatty acid released per min).

In order to determine whether apoE is required for the activation of LPL in vitro, we performed LPL activity assays with purified human LPL (hLPL). To produce purified human LPL, we have cloned the LPL cDNA in the expression vector pEE14 and transfected the construct into CHO-K1 cells (20). Human LPL secreted into the medium was purified by heparin–agarose affinity chromatography and analyzed for purity and enzymatic activity. For the activation of LPL a source of apoCII is required and this was supplied by heat-inactivated mouse plasma. LPL activity was measured by incubating 480 ng of purified human LPL with 400 μl of a triglyceride emulsion containing 5.0 μCi/ml glycerol tri[9,10-^{3}H]oleate, 0.60 mg/ml phosphatidylcholine, 5.0 mg/ml nonradioactive triolein, 0.11 m NaCl, 5.5 mg/ml albumin, 0.178 m Tris-HCl buffer (pH 8.2) and 5% (v/v) of heat-inactivated plasma from either wild-type or apoE-deficient mice at 37°C for 1 h. At the end of the incubation, 200 μl aliquots from each reaction mixture were extracted with 3.25 ml of methanol–chloroform–heptane 1.41:1.25:1.0 (v/v/v) and 1.05 ml of 50 mm carbonate/borate buffer (pH 10.5) and the aqueous phase was separated by centrifugation (2500 rpm in a swing-out rotor in an IEC Centra 7R centrifuge for 20 min). The radioactivity released into the aqueous phase was measured in a scintillation counter for 1 min and the specific activity was calculated. For certain experiments purified human apoE (from 0.65 ng to 6.50 ng) was added to the reaction mixture prior to incubation.

Plasma lipid and lipoprotein analyses

Blood samples collected from individual mice were analyzed for total cholesterol and triglycerides using enzymatic assays (Sigma Diagnostics). Lipoproteins were isolated by fast protein liquid chromatography (FPLC) using a Beckman System Gold HPLC/FPLC system with two Superose 6 columns (Pharmacia Biotechnology Inc.) connected in series (21). Plasma samples (200 μl) were injected into the FPLC and eluted with 1 mm EDTA, 154 mm NaCl, and 0.02% NaN_3 (pH 8.2). Fractions 1 to 40 (0.5 ml each) were collected and assayed for cholesterol and triglycerides (Sigma Diagnostics). Three major lipoprotein peaks were identified corresponding to mouse VLDL/chylomicron remnants (VLDL/CMR), LDL/IDL and HDL. For the determination of VLDL/CMR apolipoprotein composition, 100-μl plasma samples were centrifuged at 100,000 *g* in an Airfuge (A-100, Beckman) for 2.5 h (22). Apolipoprotein profiles were analyzed by SDS-polyacrylamide gel (4–15%) electrophoresis and visualized by Coomassie R250 staining.

In vitro studies of VLDL/CMR hydrolysis

VLDL/CMR was isolated from plasma samples pooled from 6 wild-type (FVB), or 6 apoE-deficient mice by sequential ultracentrifugal flotation at 4°C in a Beckman 70.1 Ti rotor for 20 h (23). VLDL/CMR fractions were assayed for cholesterol, triglycerides (Sigma Diagnostics), and protein content (Bio-Rad protein assay). Isolated VLDL/CMR samples were incubated with 720 ng of purified human LPL, 5 μl heat-inactivated plasma (source of apoC-II) at 37°C for 1 h. VLDL/CMR fractions prior to incubation and 1 h after incubation were re-isolated by FPLC. In each case a single peak corresponding to VLDL/CMR was identified. The re-isolated VLDL/CMR fractions were analyzed for cholesterol, triglyceride, and protein content.

Injection of mice with replication-defective adenovirus containing the human LPL cDNA

Human LPL cDNA (1.8 kb) was subcloned into the pAvCvSv shuttle plasmid vector that contained the human cytomegalovi-

rus promoter, translation enhancer sequence and SV40 polyadenylation signal as previously described by Kobayashi et al. (24). The pAvCvSv shuttle vector containing the full-length hLPL cDNA was cotransfected with pJM17 into human embryonic kidney cells (25). After propagation of the recombinant virus (Ad.hLPL) in 293 cells and screening for LPL sequences by PCR, the virus was purified by cesium chloride density ultracentrifugation, titered, and amplified (26). As a control, a recombinant adenoviral construct containing the luciferase gene (Ad.Luc) was generated using similar methodology.

Prior to Ad.hLPL injection, male wild-type and apoE-deficient mice were fasted 4–6 h and bled from the tail vein. VLDL/CMR fractions were isolated by sequential ultracentrifugal flotation at 4°C in a Beckman 70.1 Ti rotor for 20 h (23). Plasma and VLDL/CMR samples were assayed for total cholesterol and triglycerides (Sigma Diagnostics). Seven days later the same mice were injected with 2×10^9 plaque-forming units (pfu) of the purified recombinant virus stock diluted with phosphate-buffered saline. On day 9 after the injection of Ad.hLPL, mice were fasted for 4–6 h and plasma samples were collected. Plasma samples and isolated VLDL/CMR fractions were assayed for cholesterol and triglycerides (Sigma Diagnostics).

Statistical analysis

Differences in plasma lipids, lipoproteins, and LPL activities were determined using a *t*-test for unpaired samples. The method of least squares was used to calculate correlation coefficients.

RESULTS

Plasma lipid concentrations and LPL activities in wild-type and apoE-deficient mice

It is well documented that apoE-deficient mice have markedly elevated plasma cholesterol concentrations but only moderately increased plasma triglyceride concentrations compared to wild-type mice (17). In the present experiment, the plasma cholesterol level of apoE-deficient mice (578.8 ± 33.0 mg/dl, n = 30) was 5.1-fold higher than that of wild-type, FVB mice (113.0 ± 3.5 mg/dl, n = 30). Plasma triglyceride concentration of apoE-deficient mice (208.1 ± 16.8 mg/dl, n = 30) was only 1.3-fold higher than that of wild-type mice (166.4 ± 7.6 mg/dl, n = 30). FPLC fractionation of plasma lipoproteins showed that it was the VLDL/CMR fraction that was significantly elevated in apoE-deficient mice (**Fig. 1**). ApoE-deficient mice had 42-fold higher VLDL/CMR cholesterol (655.2 ± 57.1 mg/dl, n = 36) than wild-type mice (15.7 ± 1.2 mg/dl, n = 36, **Table 1**). VLDL/CMR triglyceride concentration was 2-fold higher in apoE-deficient (172.8 ± 17.3 mg/dl, n = 36) than in wild-type mice (74.5 ± 6.4 mg/dl, n = 36, Table 1).

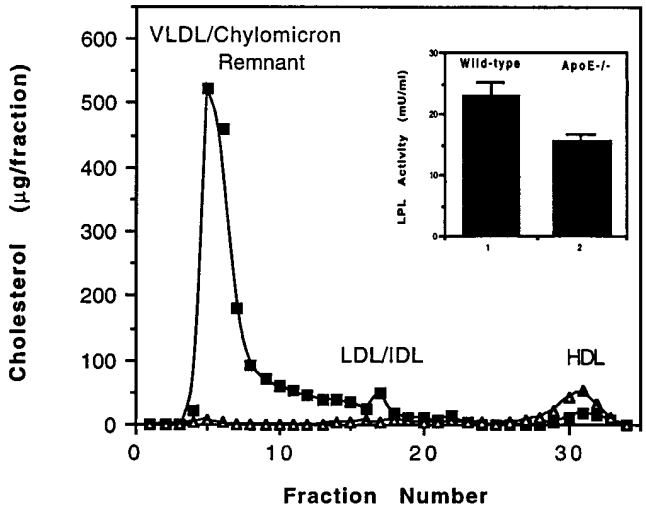

Fig. 1. FPLC profiles and LPL activities of plasma from wild-type (△) and apoE-deficient (■) mice. Plasma samples (200 μl) were separated by gel filtration chromatography using a Beckman System Gold HPLC/FPLC system with two Superose 6 columns connected in series. FPLC fractions 1–40 were collected and VLDL/CMR, LDL/IDL, and HDL fractions were identified. Cholesterol concentrations were measured in each fraction using an enzymatic assay (Sigma Diagnostics). Graphs show representative FPLC profiles of lipoproteins isolated from wild-type and apoE-deficient mice. Inset: LPL activities in 20 μl of plasma from wild-type and apoE-deficient mice. Bars represent mean ± SD, n = 36.

In view of the significant differences in VLDL/CMR concentrations and the potential role of apoE in the LPL-mediated hydrolysis of VLDL/CMR, we measured plasma LPL activities in apoE-deficient and wild-type FVB mice. Circulating LPL activity was only modestly lower in the plasma of apoE-deficient (15.5 ± 1.3 mU/ml, n = 36) mice than in the plasma of wild-type mice (22.9 ± 2.5

TABLE 1. Effect of heparin injection on plasma lipoprotein concentrations in wild-type and apoE-deficient mice

	VLDL/Chylomicron Remnants		LDL/IDL		HDL	
	Cholesterol	Triglyceride	Cholesterol	Triglyceride	Cholesterol	Triglyceride
	mg/dl		*mg/dl*		*mg/dl*	
Wild-type						
Pre-injection	15.7 ± 1.2	74.5 ± 6.4	36.0 ± 2.0	21.2 ± 2.3	142.3 ± 8.2	13.9 ± 1.8
+ Heparin	1.4 ± 0.4[b]	0.3 ± 0.2[b]	35.9 ± 3.0	26.3 ± 4.5	173.2 ± 5.0[a]	24.1 ± 3.3[a]
APOE$^{-/-}$						
Pre-injection	655.2 ± 57.1	172.8 ± 17.3	209.7 ± 9.4	28.4 ± 2.8	71.3 ± 3.5	6.0 ± 0.9
+ Heparin	901.7 ± 52.2[a]	160.5 ± 19.2	146.4 ± 10.3[b]	42.3 ± 4.0[a]	78.7 ± 5.2	26.8 ± 3.0[b]

Plasma was collected from mice after a 4–6-h fast and VLDL/chylomicron remnants, LDL/IDL, and HDL fractions were isolated by FPLC. Cholesterol and triglyceride concentrations were measured in FPLC fractions 1–40. Values represent mean ± SD, n = 36. The mice used differ from those in Fig. 2.

[a] $P < 0.05$.

[b] $P < 0.001$.

mU/ml, n = 36, $P < 0.05$, Fig. 1, inset). It is unlikely that this difference is due to mouse strain, as wild-type mice with the C57BL/6 genetic background had similar plasma LPL activity as FVB mice (data not shown).

Effect of heparin injection on plasma lipids, lipoproteins, and LPL activities in wild-type and apoE-deficient mice

Heparin releases LPL into the circulation and hence promotes the rapid hydrolysis of VLDL/CMR. In wild-type mice, the injection of heparin resulted in a 50% reduction in total plasma triglycerides ($P < 0.001$, n = 30, **Fig. 2B**) and 99% reduction in VLDL/CMR triglycerides ($P < 0.001$, n = 36, Table 1). Total plasma cholesterol concentrations (Fig. 2A) were not affected by heparin treatment, but VLDL/CMR cholesterol was reduced by 91% after heparin injection ($P < 0.001$, n = 36, Table 1). In contrast, apoE-deficient mice showed an apparent resistance to heparin. Both total plasma triglyceride (Fig. 2B) and VLDL/CMR triglyceride concentrations (Table 1) were unaffected by the injection of heparin. Post-heparin lipase activity was ~43% lower in apoE-deficient mice (38.6 ± 3.0 mU/ml, n = 37) than in wild-type mice (68.1 ± 3.8 mU/ml, n = 33, $P < 0.01$). Although this moderately lower lipase activity of apoE-deficient mice compared to wild-type mice may play a minor role in the apparent resistance of apoE-deficient VLDL/CMR to heparin-induced lipolysis, it is unlikely that it accounts for the complete absence of effect of heparin treatment in apoE-deficient mice.

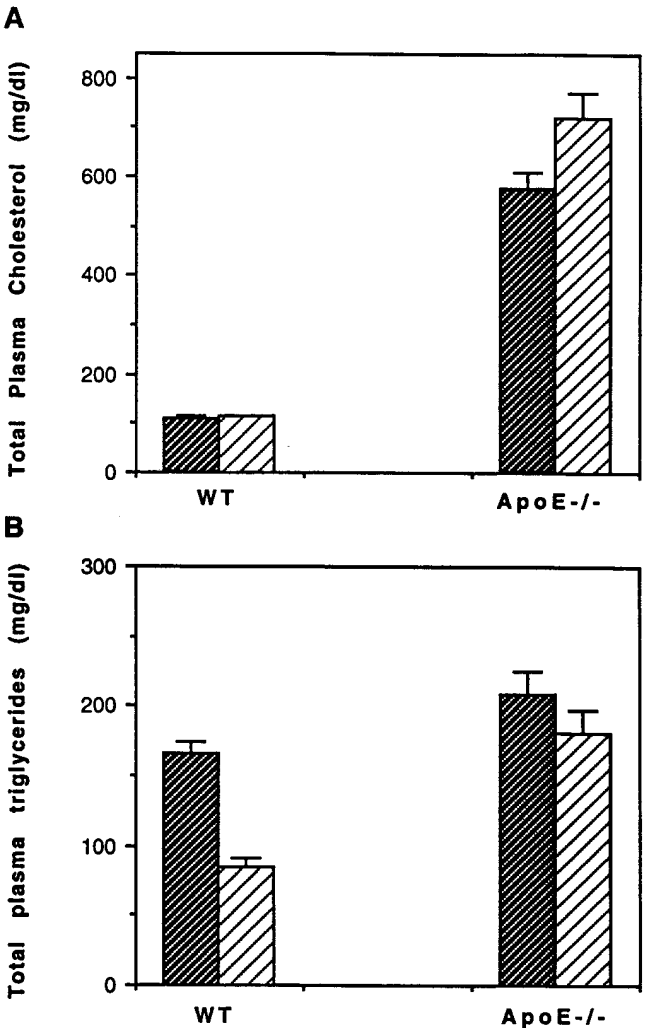

Fig. 2. Effect of heparin injection on plasma cholesterol and triglyceride concentrations of wild-type and apoE-deficient mice. Anesthetized wild-type and apoE-deficient mice were bled 5 min after intravenous injection of saline (no heparin) (▨), or 100 U/kg body weight of heparin (+ heparin) (▨). Total plasma cholesterol (A) and triglyceride concentrations (B) were measured using an enzymatic assay (Sigma Diagnostics). Bars represent mean ± SD, n = 30. The mice used differ from those in Table 1.

The effect of heparin injection on plasma VLDL/CMR, LDL/IDL, and HDL concentrations in wild-type and apoE-deficient mice is summarized in Table 1. In wild-type mice, concomitant with the virtual elimination of VLDL/CMR after heparin injection, there was a significant increase in HDL concentrations. Heparin injection increased LDL/IDL and HDL triglyceride concentrations in apoE-deficient mice, without significantly reducing VLDL/CMR concentrations.

The injection of heparin also affected the VLDL/CMR apolipoprotein profiles of wild-type mice. As judged by SDS-polyacrylamide gel electrophoresis, apoB-48 and apoE were significantly reduced in post-heparin plasma compared to pre-heparin levels (**Fig. 3**). The apoB-48 bands in pre- and post-heparin plasma from apoE-deficient mice were not significantly different (Fig. 3).

Relationship between plasma lipoprotein concentrations and lipolytic activities in wild-type and apoE-deficient mice

We examined the relationship between post-heparin LPL activity and VLDL/CMR, LDL/IDL, and HDL concentrations in wild-type and apoE-deficient mice. In wild-type mice, VLDL/CMR concentration in post-heparin plasma was close to 0, hence it was not possible to correlate it to LPL activity (**Fig. 4A**). LPL activity of wild-type mice was positively correlated with LDL cholesterol ($r = 0.53$, $P < 0.002$, n = 32, Fig. 4B) and HDL cholesterol ($r = 0.50$, $P < 0.003$, n = 32, Fig. 4C). In apoE-deficient mice, no relationships were seen between LPL activities and VLDL cholesterol ($r = 0.14$, NS, n = 32, Fig. 4D) and HDL cholesterol concentrations ($r = 0.00$, NS, n = 32, Fig. 4F). We found a positive correlation between LPL activity and LDL cholesterol concentrations in apoE-deficient mice ($r = 0.46$, $P < 0.006$, n = 32, Fig. 4E). Total plasma cholesterol levels positively correlated with LPL activity in wild-type mice ($r = 0.58$, $P < 0.001$, n = 32), but not in apoE-deficient mice ($r = 0.23$, NS, n = 32, data not shown). For both wild-type and apoE-deficient mice, the relationship between LPL activities and lipoprotein triglyceride concentrations (data not shown) was similar to what we observed for lipoprotein cholesterol (Fig. 4A–F).

Role of apoE in the activation of purified human LPL in vitro

We assayed the activity of purified human LPL (hLPL) in vitro, by incubating LPL with a ^{3}H-labeled triglyceride/

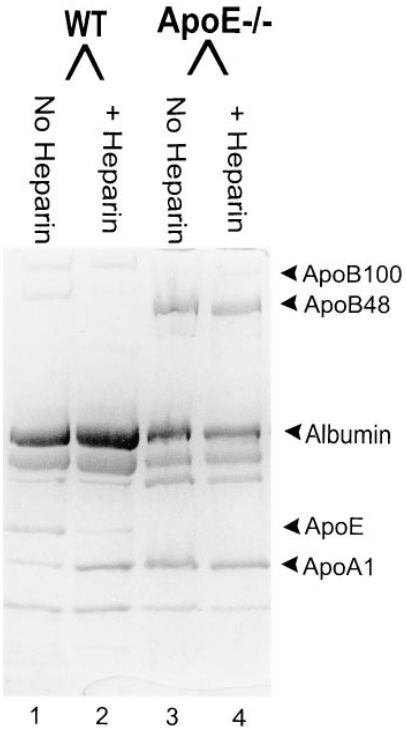

Fig. 3. Effect of heparin injection on plasma apolipoprotein composition of wild-type and apoE-deficient mice. Anesthetized wild-type and apoE-deficient mice were bled 5 min after intravenous injection of saline (no heparin), or 100 U/kg body weight of heparin (+ heparin). VLDL/CMR fractions were isolated from the plasma of wild-type and apoE-deficient mice by sequential flotation ultracentrifugation using an Airfuge (A-100, Beckman). Apolipoproteins were separated on a SDS-polyacrylamide gel (4–15%) and stained with Coomassie R250.

phospholipid emulsion in the presence or absence of heat-inactivated plasma from wild-type or apoE-deficient mice. Plasma provides apoC-II, an obligatory cofactor for LPL activity; in the absence of this protein, purified human LPL had activity close to 0. The dose of plasma required to fully activate LPL was determined by assaying LPL activity in the presence of different volumes of plasma. When LPL activity measured in the presence of 5 μl of plasma was set at 100%, the addition of 10, 20, 30, and 50 μl of plasma resulted in 85 ± 5%, 99 ± 5%, 110 ± 12%, and 103 ± 1% activation, respectively, of LPL. We concluded that 5 μl of plasma was sufficient to fully activate hLPL. Plasma from wild-type and apoE-deficient mice was equally effective in activating LPL (415.0 ± 82.3 mU/ml and 433.0 ± 35.5 mU/ml, respectively, n = 2, average ± range). Similarly, VLDL/CMR isolated from apoE-deficient mice efficiently activated purified hLPL.

In vitro hydrolysis of VLDL/CMR isolated from wild-type and apoE-deficient mice by purified hLPL

VLDL/CMR isolated from the plasma of wild-type or apoE-deficient mice by sequential flotation ultracentrifugation was re-isolated by FPLC before (pre-incubation) and after a 1-h incubation with hLPL (post-incubation with hLPL). Heat-inactivated mouse plasma (5 μl) was included in the incubation reaction to provide a standard amount of apoC-II. For VLDL/CMR isolated from wild-type mice, incubation with purified hLPL resulted in an 85% and 82% decrease in the cholesterol and triglyceride content, respectively, of the re-isolated VLDL/CMR fraction (**Fig. 5A**). VLDL/CMR concentrations did not diminish when VLDL/CMR was incubated for 1 h with heat-inactivated serum in the absence of hLPL (data not shown). The decrease in VLDL/CMR after incubation with hLPL was the same whether heat-inactivated plasma from wild-type or apoE-deficient mice was used (data not shown).

When we incubated VLDL/CMR isolated from the plasma of apoE-deficient mice with hLPL and heat-inactivated plasma, there were no significant differences between pre-incubation and post-incubation VLDL/CMR cholesterol (415.0 μg vs. 425.6 μg) and triglyceride concentrations (95.6 μg vs. 92.8 μg, Fig. 5B). The addition of purified apoE (0.6 μg to 6.5 μg) to the incubation did not have an effect on LPL-mediated lipolysis of VLDL/CMR (data not shown). Compared with wild-type VLDL/CMR particles, VLDL/CMR isolated from apoE-deficient mice have significantly higher cholesterol content, but similar amounts of triglycerides. The protein content of apoE-deficient VLDL/CMR was ~3- to 4-fold higher than that of VLDL/CMR from wild-type mice. In order to approximate the ratio of apoE-deficient VLDL/CMR protein to LPL enzyme to that of wild-type mice, we reduced the amount of apoE-deficient VLDL/CMR in the reaction mixture by ~4-fold. When we repeated the incubation of purified hLPL with the reduced amount of VLDL/CMR from apoE-deficient mice, VLDL/CMR particles were hydrolyzed (Fig. 5C) to a similar extent as VLDL/CMR from wild-type mice (Fig. 5A). The cholesterol and triglyceride contents of the re-isolated VLDL/CMR fractions from apoE-deficient mice were reduced by 91% and 94%, respectively, after incubation with purified hLPL (Fig. 5C).

Effect of Ad.hLPL injection on plasma cholesterol and triglyceride concentrations of wild-type and apoE-deficient mice

Overexpression of hLPL in apoE-deficient mice was induced by intravenous injection of a replication-defective adenoviral vector containing a hLPL cDNA driven by a cytomegalovirus promoter. Adenovirus-mediated overexpression of hLPL resulted in significant reductions in plasma cholesterol (from 137.88 ± 13.01 mg/dl to 65.03 ± 7.34 mg/dl, n = 4) and triglyceride concentrations (from 164.65 ± 15.24 mg/dl to 47.49 ± 3.83 mg/dl, n = 4) in wild-type mice (**Fig. 6**). VLDL cholesterol and triglyceride concentrations were also significantly lower on day 9 after transduction with Ad.hLPL (Fig. 6). Injection of the control vector Ad.Luc did not significantly affect plasma lipid concentrations (27). Although plasma cholesterol concentrations were markedly elevated in apoE-deficient mice, transduction with Ad.hLPL reduced total plasma cholesterol (from 936.29 ± 268.41 mg/dl to 263.92 ± 45.78 mg/dl, n = 3) and triglycerides (from 236.54 ± 88.21 mg/dl to 30.21 ± 9.05 mg/dl, n = 3). Similarly, VLDL cholesterol and triglycerides were reduced by 91%

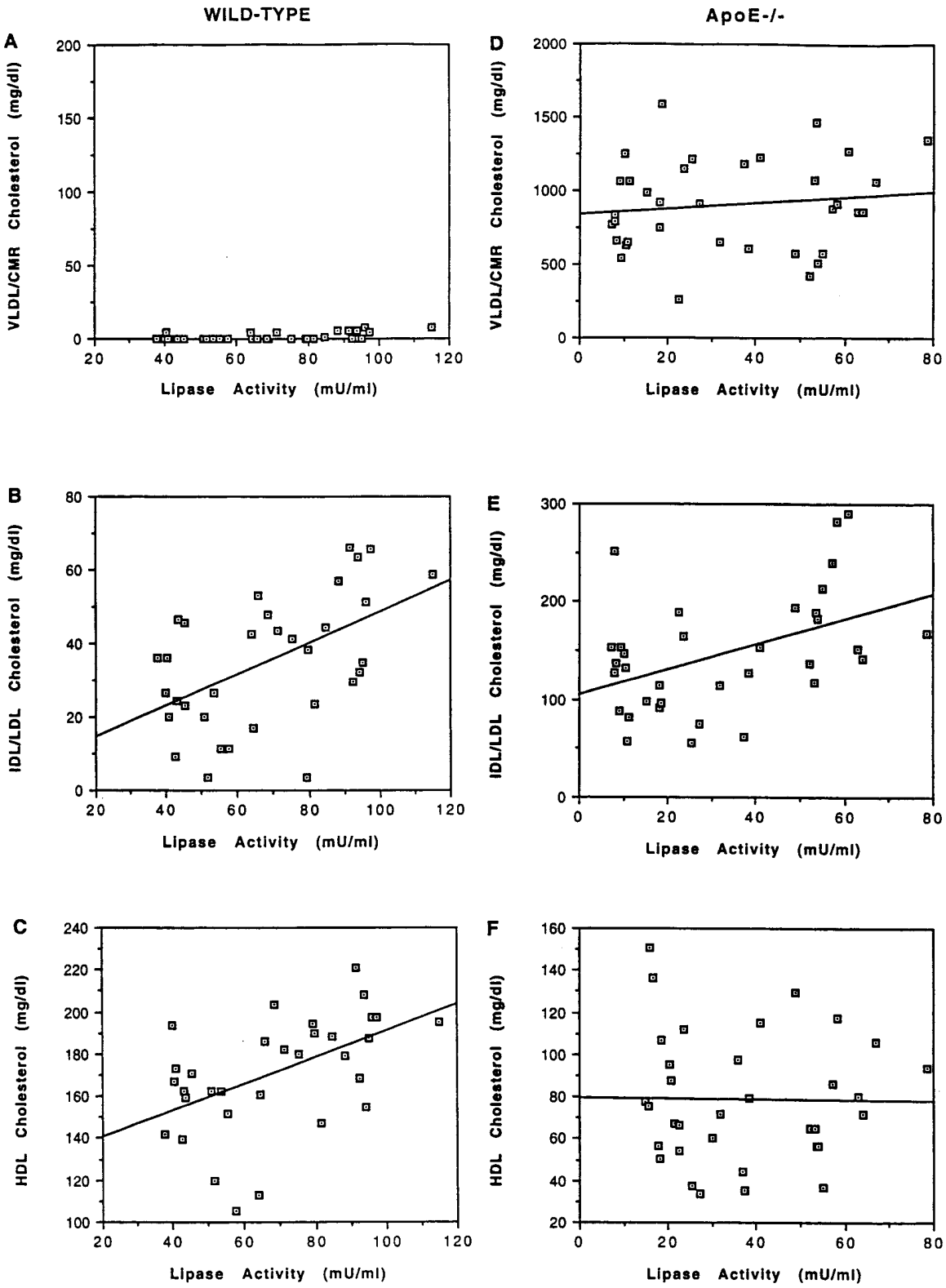

Fig. 4. Relationship between lipoprotein cholesterol concentrations and post-heparin lipase activities. VLDL/CMR, LDL/IDL, and HDL fractions were isolated from 200 μl of plasma from wild-type and apoE-deficient mice. Cholesterol concentrations were measured in each fraction using an enzymatic assay (Sigma Diagnostics). Lipase activities were assayed in 20 μl of post-heparin plasma obtained 5 min after intravenous injection of heparin (100 U/kg body weight) into wild-type and apoE-deficient mice. VLDL/CMR, LDL/IDL, and HDL concentrations (mg/dl) were plotted against corresponding lipase activities (mU/ml) for 32 individual mice. Correlation coefficients (r) were calculated by using the method of least squares.

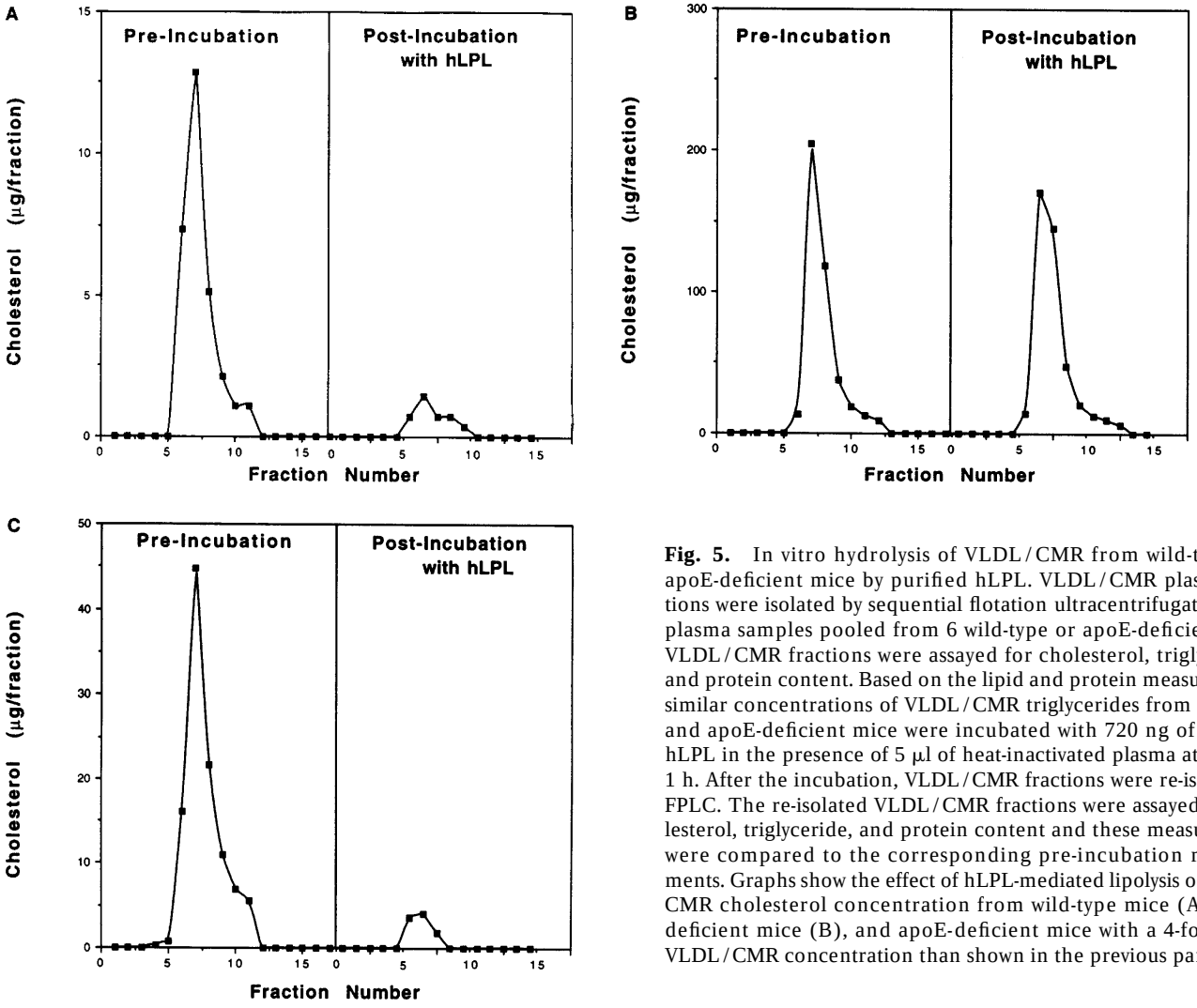

Fig. 5. In vitro hydrolysis of VLDL/CMR from wild-type and apoE-deficient mice by purified hLPL. VLDL/CMR plasma fractions were isolated by sequential flotation ultracentrifugation from plasma samples pooled from 6 wild-type or apoE-deficient mice. VLDL/CMR fractions were assayed for cholesterol, triglycerides, and protein content. Based on the lipid and protein measurements, similar concentrations of VLDL/CMR triglycerides from wild-type and apoE-deficient mice were incubated with 720 ng of purified hLPL in the presence of 5 μl of heat-inactivated plasma at 37°C for 1 h. After the incubation, VLDL/CMR fractions were re-isolated by FPLC. The re-isolated VLDL/CMR fractions were assayed for cholesterol, triglyceride, and protein content and these measurements were compared to the corresponding pre-incubation measurements. Graphs show the effect of hLPL-mediated lipolysis on VLDL/CMR cholesterol concentration from wild-type mice (A), apoE-deficient mice (B), and apoE-deficient mice with a 4-fold lower VLDL/CMR concentration than shown in the previous panels (C).

and 93%, respectively, after Ad.hLPL injection (Fig. 6). Therefore, induced overexpression of LPL activity overcame the apparent resistance of the apoE-deficient lipoproteins to LPL-mediated lipolysis.

DISCUSSION

Although numerous in vitro studies have examined the effect of apoE on LPL activity (reviewed in ref. 2), the in vivo role of apoE in the regulation of lipolysis is unknown. Our approach to studying the mechanism of lipolysis and specifically the role of apoE in this process was to use the apoE-deficient mouse model. We present both in vitro and in vivo evidence that there is no absolute requirement for apoE in the activation of LPL. Furthermore, we show that in addition to compositional factors that affect the susceptibility of lipoprotein particles to LPL-mediated lipolysis, efficient lipolysis also depends on the relative concentrations of enzyme to lipoprotein substrate.

Mice deficient in apoE, the ligand that clears lipoproteins via the LDL receptor and LDL-related protein (LRP) in the liver, have markedly elevated plasma cholesterol concentrations and the mice develop spontaneous atherosclerosis (17, 18). Compared to wild-type mice, apoE-deficient mice have a significant accumulation of VLDL and chylomicron remnant lipoprotein particles in the VLDL size range. We found that although mice lacking apoE have only modestly lower LPL activities than wild-type mice (Fig. 1), the injection of heparin, which releases membrane-bound LPL into the circulation, did not result in any reduction in plasma triglyceride levels (Fig. 2). Unlike in wild-type mice, VLDL/CMR concentrations (Table 1), as well as apolipoprotein profiles of apoE-deficient mice (Fig. 3), remained unaffected by the injection of heparin. Similarly, when VLDL/CMR particles isolated from equal plasma volumes from wild-type and apoE-deficient mice were treated with the same amount of purified human LPL in vitro, the VLDL/CMR from wild-type mice was almost completely hydrolyzed, whereas the apoE-deficient VLDL/CMR was apparently resistant to hydrolysis (Fig. 5A and B). This apparent resistance of apoE-deficient VLDL/CMR to LPL-mediated lipolysis led us to examine the relationship of plasma lipoproteins and LPL activity in wild-type and apoE-deficient mice.

ApoE-deficient mice differ from wild-type mice substan-

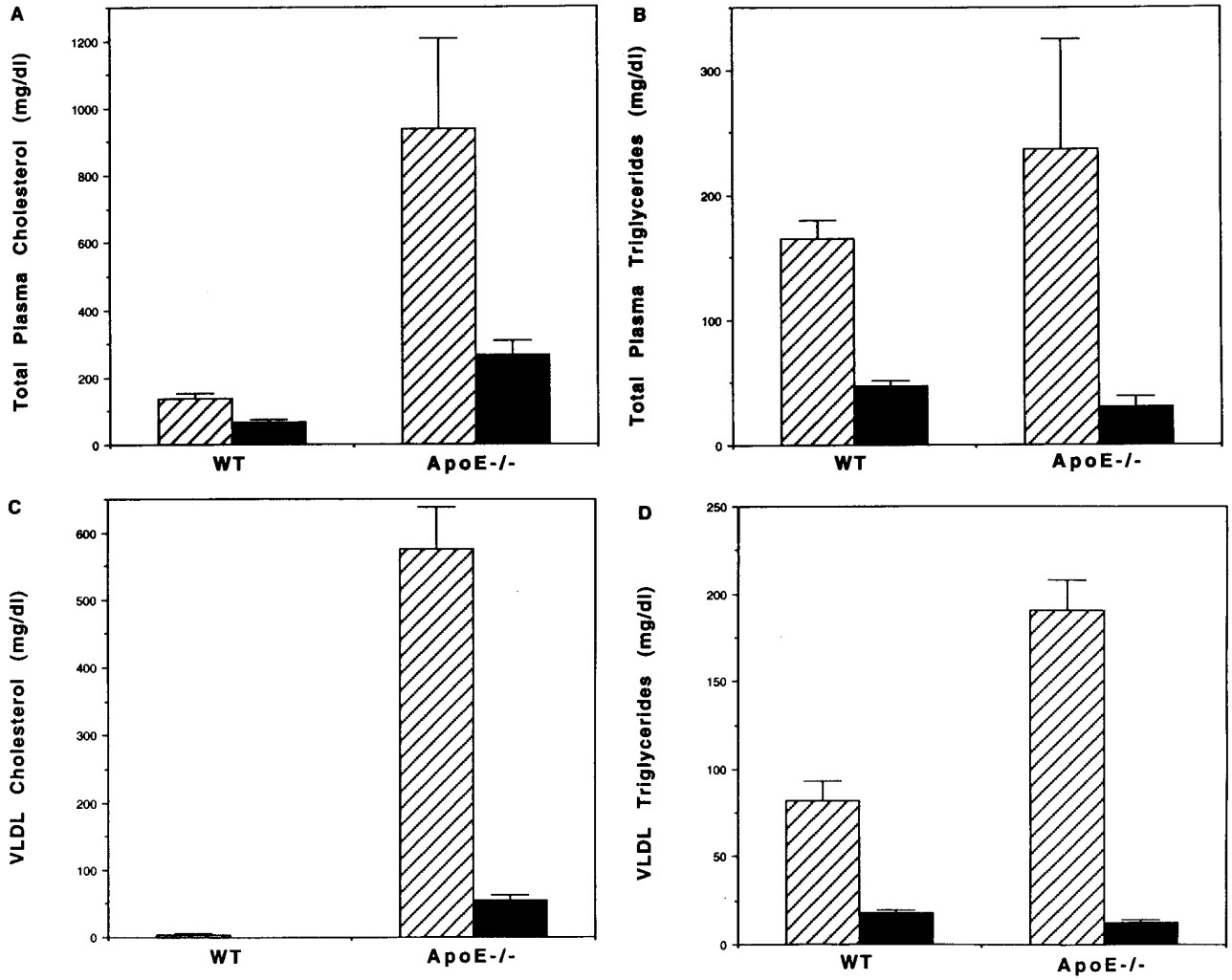

Fig. 6. Plasma cholesterol and triglyceride concentrations of wild-type and apoE-deficient mice injected with Ad.hLPL. A replication-defective adenoviral vector containing hLPL cDNA driven by a cytomegalovirus promoter (Ad.hLPL) was injected into the jugular vein of anesthetized wild-type and apoE-deficient mice. Mice were fasted for 4–6 h prior to bleeding them from the tail vein. VLDL/CMR lipoprotein fractions were isolated by sequential ultracentrifugal flotation. Graphs show total plasma cholesterol (A), total plasma triglyceride (B), VLDL/CMR cholesterol (C), and VLDL/CMR triglyceride concentrations (D) measured before (pre-injection) (▨), and 9 days after (Ad.hLPL) (■), the injection of Ad.hLPL. Mean ± SD, n = 4 for wild-type and n = 3 for apoE-deficient mice.

tially in the relationship between LPL activity and lipoprotein concentrations. In wild-type mice, concomitant with the disappearance of VLDL/CMR triglycerides from post-heparin plasma, LPL activity showed a strong positive correlation with plasma LDL and HDL concentrations (Fig. 4). This relationship was reminiscent of previous studies in humans, where LPL activity was negatively correlated with plasma triglycerides and showed a strong positive relationship with HDL cholesterol concentrations (28–32). Unlike in wild-type mice and in humans, VLDL/CMR and HDL concentrations in apoE-deficient mice did not vary with LPL activity.

The mechanism of lipolysis is not fully understood and there may be a number of possible explanations for the observed differences in LPL-mediated catabolism of VLDL/CMR particles in apoE-deficient mice. In our studies apoE-deficient mice had modestly lower LPL activity than wild-type mice, but it is unlikely that apoE is an important co-factor or activator of LPL as in vitro lipolysis assays show that apoE-deficient plasma is equally effective as plasma from wild-type mice in activating LPL. Furthermore, the addition of an excess amount of purified apoE has no stimulatory, or inhibitory, effect on LPL activity. This observation does not preclude, however, some subtle role for apoE in lipolysis in vivo. It has been suggested previously that the presence of apoE is required for successful interaction of LPL with its lipoprotein substrates (reviewed in ref. 2) and in the absence of this apolipoprotein, lipoprotein particles may not successfully dock in the vicinity of LPL and hence the enzyme–substrate interaction may be compromised.

A possible explanation for the apparent resistance to heparin-induced lipolysis of VLDL/CMR from apoE-deficient mice may be that these lipoprotein particles have certain compositional properties that make them less favorable substrates for LPL. Normally, triglyceride-rich

lipoproteins that are hydrolyzed by LPL have very low cholesterol content. In contrast, VLDL particles from apoE-deficient mice are high in cholesterol and this unusual composition of the particles may result in conformational changes that could affect lipolysis. ApoC-II, associated with lipoprotein substrates, is a cofactor for LPL and changes in the composition of lipoprotein, such as cholesterol enrichment, may affect the accessibility of apoC-II to the enzyme and hence prevent efficient lipolysis. VLDL/CMR from apoE-deficient mice also have significantly different apolipoprotein profiles compared to lipoprotein particles from wild-type mice (18, 19). VLDL/CMR particles isolated from apoE-deficient mice have elevated apoB-48, apoA-I, and apoA-IV concentrations. The in vivo role of these apolipoproteins in LPL-mediated lipolysis is unclear, but in vitro studies have shown that apoA-I has no effect on LPL, whereas apoA-IV activates LPL (33). The authors suggest that apoA-IV may be involved in the release of apoC-II from VLDL or HDL particles and this in turn may facilitate apoC-II interactions with LPL. ApoC-III concentrations may also modulate lipolysis, as it has been reported that the activation of LPL by apoC-II is noncompetitively inhibited by apoC-III (7). It has also been suggested that apoB may be important for the interaction of lipoproteins with LPL (34). LPL-mediated lipolysis is a complex process and the unusual apolipoprotein profiles of apoE-deficient mice may contribute to the observed differences in VLDL/CMR lipolysis. We must also consider the possibility that VLDL/CMR fractions isolated from the plasma of apoE-deficient mice are not strictly nascent lipoprotein particles, but contain partial lipolysis products that may have an inhibitory effect on LPL activity. Connelly et al. (35) have demonstrated that cholesterol-rich VLDL and IDL particles are potent inhibitors of LPL and it is possible that in our studies LPL activity in apoE-deficient mice is also partially inhibited by lipolysis products.

Alternatively, or additionally, marked elevation in the VLDL/CMR fraction of apoE-deficient mice may simply saturate the lipolysis machinery. The importance of VLDL particle number on LPL-dependent lipolysis has been previously reported (35). Lipolysis has been referred to as the common saturable pathway as different triglyceride-rich lipoproteins compete for a common enzyme (36). A similar situation may exist in patients with severe hypertriglyceridemia where LPL cannot cope with the massive influx of substrate particles. We decided to test whether the apparent resistance of LPL-mediated lipolysis of triglyceride-rich lipoproteins from apoE-deficient mice may be overcome by increasing LPL concentrations relative to substrate concentrations in vitro and in vivo.

For VLDL/CMR triglyceride concentrations similar to wild-type mice, VLDL/CMR particles from apoE-deficient mice have several-fold higher cholesterol and protein concentrations. In vitro LPL assays showed that by adjusting the amount of apoE-deficient VLDL/CMR substrate to LPL protein to ratios similar to those observed in wild-type mice, we achieved almost complete hydrolysis of VLDL/CMR from apoE-deficient mice (Fig. 5C). Our observation that the apparent resistance of VLDL/CMR from apoE-deficient mice may be overcome by altering the relative concentration of substrate particles to enzyme was also supported by in vivo studies. Adenovirus-mediated transfer of genes is an effective method to induce high levels of expression of specific proteins in vivo (37). We have previously shown that adenovirus-mediated gene transfer into mice results in very high levels of liver-specific LPL expression, significant elevation in post-heparin plasma LPL activity and enhancement of LPL-mediated clearance of lipoprotein particles (27). Using this technique of LPL gene delivery to apoE-deficient mice, we were able to achieve marked reduction in VLDL/CMR cholesterol and virtual elimination of VLDL/CMR triglycerides (27, Fig. 6). The mechanism by which Ad.hLPL injection results in a reduction of plasma VLDL/CMR concentrations is unclear. It is possible that VLDL/CMR particles are cleared by LPL-mediated hydrolysis, but as liver-specific overexpression of LPL represents a nonphysiological situation, enhanced hepatic clearance of lipoprotein particles may also occur. In this regard it has been previously documented that LPL directly facilitates the interaction between lipoproteins and various receptors and hence promotes the clearance of the entire lipoprotein particles (38–44). The ligand and/or receptors involved in the proposed LPL-dependent clearance of apoE-deficient lipoprotein particles is unknown, but the significant reduction in the VLDL/CMR apoB-48 and apoB-100 concentrations suggests apoB-mediated uptake (27). The methodology of the present study did not allow us to determine the relative extent of the clearance mediated by these two pathways. Thus, the resistance of heparin-induced lipolysis in the dyslipoproteinemia of apoE deficiency is not absolute and it may be corrected by adjusting substrate to enzyme ratio to approximate a more normal situation in vitro or by overexpressing LPL in vivo.

This study was supported by a grant (HL16512) from the National Institutes of Health. Dr. Kunihisa Kobayashi was supported by a Fellowship from the Juvenile Diabetes Foundation International. We thank Sylvia A. Ledesma for expert secretarial assistance.

Manuscript received 24 October 1997, in revised form 30 March 1998, and in re-revised form 19 May 1998.

REFERENCES

1. Austin, M. A. 1991. Plasma triglyceride and coronary heart disease. *Arterioscler. Thromb.* **11:** 2–14.
2. Goldberg, I. J. 1996. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. *J. Lipid Res.* **37:** 693–707.
3. Olivecrona, G., and T. Olivecrona. 1995. Triglyceride lipases and atherosclerosis. *Curr. Opin. Lipidol.* **6:** 291–305.
4. LaRosa, J. C., R. I. Levy, P. N. Herbert, S. E. Lux, and D. S. Fredrickson. 1970. A specific apoprotein activator for lipoprotein lipase. *Biochem. Biophys. Res. Commun.* **41:** 57–62.
5. Breckenridge, W. J., J. Little, P. Alaupovic, C. S. Wang, A. Kuksis, G. Kakis, F. Lindgren, and G. Gardiner. 1982. Lipoprotein abnormalities associated with a familial deficiency of hepatic triglyceride lipase. *Atherosclerosis.* **45:** 161–179.
6. Brown, W. V., and M. L. Baginsky. 1972. Inhibition of lipoprotein

lipase by an apoprotein of human very low density lipoprotein. *Biochem. Biophys. Res. Commun.* **46:** 375–381.
7. McConathy, W. J., J. C. Gesquiere, H. Bass, A. Tartar, J. Fruchard, and C. Wang. 1992. Inhibition of lipoprotein lipase activity by synthetic peptides of apolipoprotein C-III. *J. Lipid Res.* **33:** 995–1003.
8. Fielding, P. E., Y. Ishikawa, and C. J. Fielding. 1989. Apolipoprotein E mediates binding of normal very low density lipoprotein to heparin but is not required for high affinity. *J. Biol. Chem.* **264:** 12462–12466.
9. Ji, Z. S., W. J. Brecht, R. D. Miranda, M. M. Hussain, T. L. Innerarity, and R. W. Mahley. 1993. Role of heparan sulfate proteoglycans in the binding and uptake of apolipoprotein E-enriched remnant lipoproteins by cultured cells. *J. Biol. Chem.* **268:** 10160–10167.
10. Fazio, S., Y. L. Lee, Z. S. Ji, and S. J. Rall. 1993. Type III hyperlipoproteinemic phenotype in transgenic mice expressing dysfunctional apolipoprotein E. *J. Clin. Invest.* **92:** 1497–1503.
11. Ghiselli, G. E., E. J. Schaefer, P. Gascon, and H. B. Brewer, Jr. 1981. Type III hyperlipoproteinemia associated with apolipoprotein E deficiency. *Science.* **214:** 1239–1241.
12. Lohse, P., H. B. Brewer, III, M. S. Meng, S. I. Skarlatos, J. C. LaRosa, and H. J. Brewer, Jr. 1992. Familial apolipoprotein E deficiency and type III hyperlipoproteinemia due to a premature stop codon in the apolipoprotein E gene. *J. Lipid Res.* **33:** 1583–1590.
13. Quarfordt, S. H., M. Hilderman, M. R. Greenfield, and F. A. Shelburne. 1977. The effect of human arginine-rich apoprotein on rat adipose lipoprotein lipase. *Biochem. Biophys. Res. Commun.* **79:** 302–308.
14. Yamada, N., and T. Murase. 1980. Modulation, by apolipoprotein E, of lipoprotein lipase activity. *Biochem. Biophys. Res. Commun.* **94:** 710–715.
15. Rensen, P. C. N., and T. J. C. van Berkel. 1996. Apolipoprotein E effectively inhibits lipoprotein lipase-mediated lipolysis of chylomicron-like triglyceride-rich lipid emulsions in vitro and in vivo. *J. Biol. Chem.* **271:** 14791–14799.
16. Ekman, R., and P. Nilsson-Ehle. 1975. Effects of apolipoproteins on lipoprotein lipase activity of human adipose tissue. *Clin. Chem. Acta.* **63:** 29–35.
17. Piedrahita, J. A., S. H. Zhang, J. R. Hagaman, P. M. Oliver, and N. Maeda. 1992. Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. *Proc. Natl. Acad. Sci. USA.* **89:** 4471–4475.
18. Plump, A. S., J. D. Smith, T. Hayek, K. Aalto-Setala, A. Walsh, J. G. Verstuyft, E. M. Rubin, and J. L. Breslow. 1992. Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell.* **71:** 343–353.
19. Zhang, S. H., R. L. Reddick, J. A. Piedrahita, and N. Maeda. 1992. Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science.* **258:** 468–471.
20. Zsigmond, E., E. Scheffler, T. Forte, R. Potenz, W. Wu, and L. Chan. 1994. Transgenic mice expressing human lipoprotein lipase driven by the mouse metallothionein promoter. A phenotype associated with increased perinatal mortality and reduced plasma VLDL of normal size. *J. Biol. Chem.* **269:** 18757–18766.
21. Cole, T., R. Kitchens, A. Daugherty, and G. Schonfeld. 1988. An improved method for separation of triglyceride-rich lipoproteins by FPLC. *FPLC Bio. Commun.* **4:** 4–6.
22. Walsh, A., Y. Ito, and J. L. Bresslow. 1989. High levels of human apolipoprotein A-I in transgenic mice result in increase plasma levels of small high density lipoprotein HDL particles comparable to human HDL_3. *J. Biol. Chem.* **264:** 6488–6494.
23. Lindgren, F. T., L. C. Jensen, and F. T. Hatch. 1972. The isolation and quantitative analysis of serum lipoproteins. *In* Blood Lipids and Lipoproteins. G. J. Nelson, editor. John Wiley-Interscience, New York. 181–274.
24. Kobayashi, K., K. Oka, T. Forte, B. Y. Ishida, B-B. Teng, K. Ishimura-Oka, M. Nakamuta, and L. Chan. 1996. Reversal of hypercholesterolemia in low density lipoprotein receptor knockout mice by adenovirus-mediated gene transfer of the very low density lipoprotein receptor. *J. Biol. Chem.* **271:** 6851–6860.
25. Graham, F. L., J. Smiley, W. C. Russell, and R. Norin. 1977. Characteristics of a human cell line transformed by human adenovirus type 5. *J. Gen. Virol.* **36:** 59–72.
26. Teng, B-B., S. Blumenthal, T. Forte, N. Navaratnam, J. Scott, A. M. Gotto, Jr., and L. Chan. 1994. Adenovirus-mediated gene transfer of rat apolipoprotein B mRNA editing protein in mice virtually eliminates apolipoprotein B-100 and normal low density lipoprotein production. *J. Biol. Chem.* **269:** 29395–29404.
27. Zsigmond, E., K. Kobayashi, K-W. Tzung, L. Li, Y. Fuke, and L. Chan. 1997. Adenovirus-mediated gene transfer of human lipoprotein lipase ameliorates the hyperlipidemias associated with apolipoprotein E and LDL receptor deficiencies in mice. *Hum. Genet. Ther.* **8:** 1921–1933.
28. Glaser, D. S., T. J. Yost, and R. H. Eckel. 1992. Preheparin lipoprotein lipolytic activities: relationship to plasma lipoproteins and postheparin lipolytic activities. *J. Lipid Res.* **33:** 209–214.
29. Nikkila, E. A., M. Taskinen, and T. Sane. 1987. Plasma high-density lipoprotein concentration and subfraction distribution and relation to triglyceride metabolism. *Am. Heart J.* **113:** 543–548.
30. Burns, C. M., H. Chu, S. M. Rueter, L. K. Hutchinson, H. Canton, E. Sanders-Bush, and R. B. Emeson. 1997. Regulation of serotonin-2C receptor G-protein coupling by RNA editing. *Nature.* **387:** 303–308.
31. Jackson, R. L., M. T. Yates, C. A. McNearey, and M. L. Kashyap. 1990. Relationship between post-heparin plasma lipases, triglycerides and high density lipoproteins in normal subjects. *Horm. Metab. Res.* **22:** 289–294.
32. Taskinen, M., C. J. Glueck, M. L. Kashyap, L. Srivastava, B. A. Hynd, G. Perisutti, K. Robinson, P. J. Kinnunen, and T. Kuusi. 1980. Post-heparin plasma lipoprotein and hepatic lipase. Relationship to high density lipoprotein cholesterol and to apolipoprotein C-II in familial hyperalphalipoproteinemic and in normal subjects. *Atherosclerosis.* **37:** 247–256.
33. Goldberg, I. J., C. A. Scheraldi, L. K. Yacoub, V. Saxena, and C. L. Bisgaier. 1990. Lipoprotein apoC-II activation of lipoprotein lipase modulation by apolipoprotein A-I and A-IV. *J. Biol. Chem.* **265:** 4266–4272.
34. Choi, S. Y., P. Sivaram, D. E. Walker, L. K. Curtiss, D. G. Gretch, S. L. Sturley, A. D. Attie, R. J. Deckelbaum, and I. J. Goldberg. 1995. Lipoprotein lipase association with lipoproteins involves protein–protein interaction with apolipoprotein B. *J. Biol. Chem.* **270:** 8081–8086.
35. Connelly, P. W., G. F. Maguire, C. Vezina, R. A. Hegele, and A. Kuksis. 1994. Kinetics of lipolysis of very low density lipoproteins by lipoprotein lipase. *J. Biol. Chem.* **269:** 20554–20560.
36. Brunzell, J. D., W. R. Hazzard, D. Porte, Jr., and E. L. Bierman. 1973. Evidence for a common, saturable, triglyceride removal mechanism for chylomicrons and very low density lipoproteins in man. *J. Clin. Invest.* **52:** 1578–1585.
37. Chan, L. 1995. Use of somatic gene transfer to study lipoprotein metabolism in experimental animals in vivo. *Curr. Opin. Lipidol.* **6:** 335–340.
38. Beisiegel, U., W. Weber, and G. Bengtsson-Olivecrona. 1991. Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein. *Proc. Natl. Acad. Sci. USA.* **88:** 8342–8346.
39. Eisenberg, S., E. Sehayek, T. Olivecrona, and I. Vlodavsky. 1992. Lipoprotein lipase enhances binding of lipoproteins to heparan sulfate on cell surfaces and extracellular matrix. *J. Clin. Invest.* **90:** 2013–2021.
40. Mulder, M., P. Lombardi, H. Jansen, T. J. C. van Berkel, R. R. Frants, and L. M. Havekes. 19XX. Heparan sulphate proteoglycans are involved in the lipoprotein lipase-mediated enhancement of the cellular binding of very low density and low density lipoproteins. *Biochem. Biophys. Res. Commun.* **185:** 582–587.
41. Wilnow, T. E., J. L. Goldstein, K. Orth, M. S. Brown, and J. Herz. 1992. Low density lipoprotein receptor-related protein and gp330 bind similar ligands, including plasminogen activator-inhibitor complexes and lactoferrin, an inhibitor of chylomicron remnant clearance. *J. Biol. Chem.* **267:** 26172–26180.
42. Nykjaer, A., M. Nielsen, A. Lookene, N. Meyers, H. Roigaard, M. Etzerodt, U. Beisiegel, G. Olivecrona, and J. Gliemann. 1994. A carboxyl-terminal fragment of lipoprotein lipase binds to the low density lipoprotein receptor-related protein and inhibits lipase-mediated uptake of lipoprotein in cells. *J. Biol. Chem.* **269:** 31747–31755.
43. Williams, S. E., I. Inoue, H. Tran, G. L. Fry, M. W. Pladet, P. H. Iverius, J. M. Lalouel, D. A. Chappell, and D. K. Strickland. 1994. The carboxyl-terminal domain of lipoprotein lipase binds to the low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor (LRP) and mediates binding of normal very low density lipoproteins to I.R.P. *J. Biol. Chem.* **269:** 8653–8658.
44. Argraves, K. M., F. D. Battey, C. D. MacCalman, M. K. R. M. Gafvels, K. F. Kozarsky, D. A. Chappell, J. F. Straus III, and D. K. Strickland. 1995. The very low density lipoprotein receptor mediates the cellular catabolism of lipoprotein lipase and urokinase-plasminogen activator inhibitor type I complexes. *J. Biol. Chem.* **270:** 26550–26557.